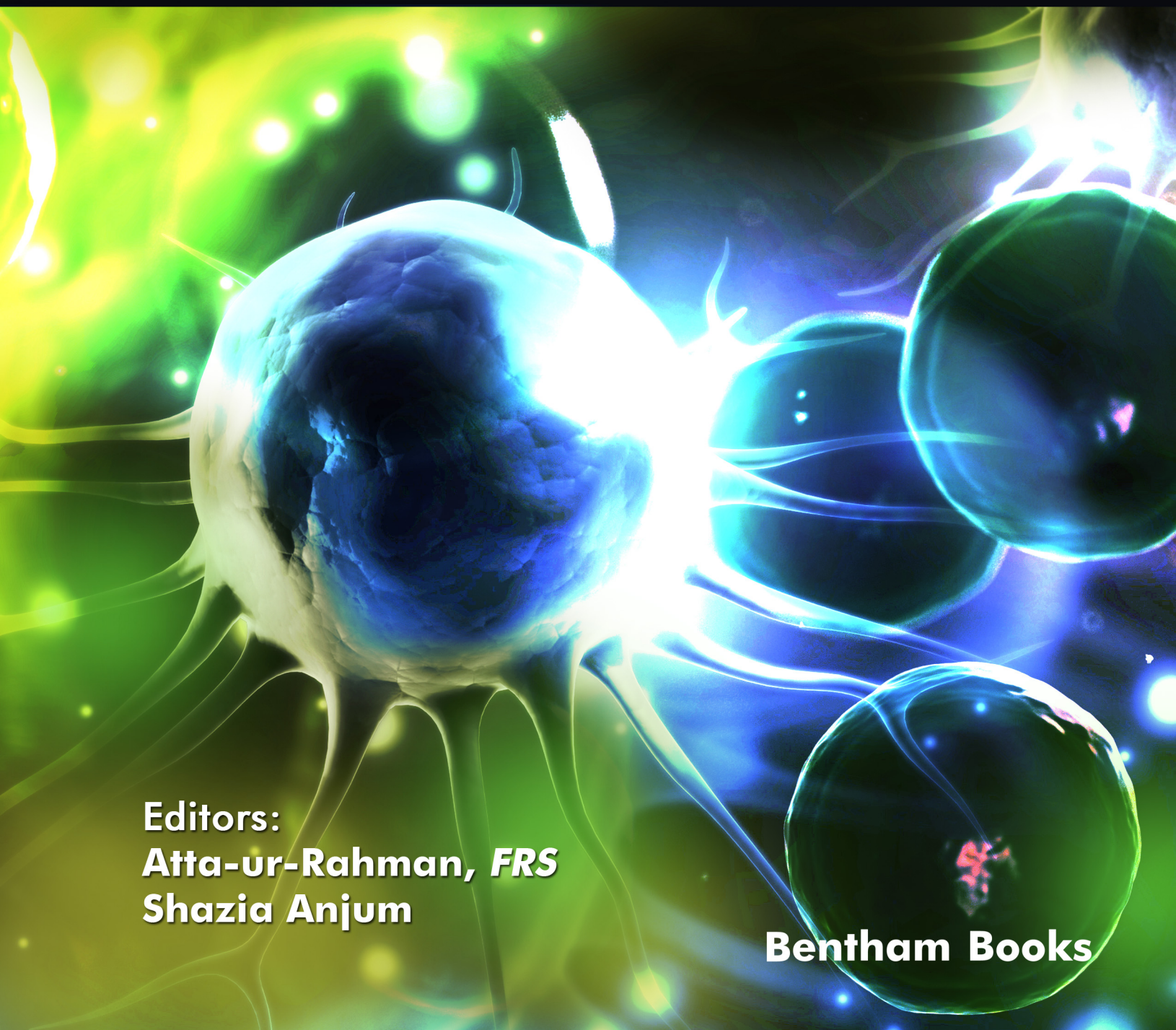
Frontiers in Stem Cell and Regenerative Medicine Research



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Frontiers in Stem Cell and Regenerative Medicine Research

(*Volume 9*)

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PREFACE

The ninth volume of 'Frontiers in Stem Cell and Regenerative Medicine Research' presents important recent developments in this fast growing field.

Hasan and Wu review the current literature on the utility of exogenous and endogenous neural stem cells in spinal cord injury. Miguel *et al*. focus on the various sources of somatic cells for human induced pluripotent stem cells (iPSC) generation, their characterization, and the progress on directed differentiation toward several cell types.

Cell signaling and redox reactions are involved in the regulation of neural-lineage cells for reprograming of adipose-derived stem cells. Abrahamse *et al*. discuss the role of reactive oxygen species (ROS) and ROS mediated cellular signaling for differentiation of stem cells.

The human skin represents the largest and most accessible part of the body that is exposed to infections, physical wounds, diseases *etc*. Titorencu *et al*. describe commercially available skin substitutes that are in demand and also described the use of stem cells for skin regeneration. Recently, the role of microRNA in cardiac conduction diseases, arrhythmogenesis and their therapeutic potential has caught the attention of researchers. Sankaranarayanan *et al*. have reviewed the role of miRNAs in cardiac rhythmic disorders and their potential in diagnosis and treatment. Finally, Rivera *et al*. discuss the use of stem cell therapy in the treatment of malaria.

We owe our special thanks to all the contributors for their valuable contribution in bringing together the ninth volume of this book series. We also thank the editorial staff of Bentham Science Publishers for their help and support.

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CHAPTER 1

Insights into Exogenous, Endogenous and Combination Therapies of Neural Stem Cells in Spinal Cord Injury

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Abstract: Advancements in the understanding of spinal cord injury (SCI) and repair have taken great leaps in the past decade. Although understanding has evolved significantly there continues to be major limitations in the clinical interventions available for patients with spinal cord injuries. Exogenous stem cells (ExNSC) have progressed to human clinical studies, but have limitations due to ethical issues, technique and long term outcomes. However, the use of ExSCs through a stimulatory effect on growth factors, cytokine production and neurotrophic factors post injury may be beneficial. Bone marrow derived stem cells, mesenchymal stem cells, embryonic stem cells, umbilical cord stem cells, adipose derived stem cells, NSCs, Schwann cells grafts and olfactory ensheathing cells have been various types of exogenous cell types and techniques used in SCIs. The role of endogenous stem cells (eNSCs) in SCI has been promising, but still requires better lineage analyses to fully understand the responses of NSCs after SCI. It has been demonstrated that there exists a bidirectional interaction within the neuro vascular system forming the neuro vascular niche. Purinergic receptor activation was found to alter the intrinsic properties of the ependymal stem/progenitor cells enhancing regulation of proliferation, differentiation and lineage specification after a SCI. Therapies have been described using nervous tissue in combination with various synthetic bridges to overcome the structural barriers of regeneration through bypassing the injured area. More recently, newer techniques such as electrical stimulation have been described to stimulate mature neuronal differentiation. Various groups have emphasized that the glial scar is counter productive. Anderson *et al.* have shown the beneficial effects of a chronic glial scar in neural tissue preservation after SCI. Moreover, they have demonstrated higher levels of chondroitin sulfate proteoglycans in injured spinal cords independent of the glial scar. In sum, we have reviewed the previous and current literature on NSC and SCI to address the neurobiological utility of NSCs in spinal cord injury.

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Keywords: Clinical Neurology, Cytokine, Electrical Stimulation, Electroacupuncture, Endogenous Neural Stem Cell, Exogenous Neural Stem Cell, Genetic Fate Mapping, Glial Scar, Growth Factor, Induced Pluripotent Stem Cell, Neural Stem Cell, Neurovascular Niche, Oligodendrocyte Ensheathing Cells, Progenitor, Purinergic Receptors, Schwann Cell Transplantation, Spinal Cord Injury, Stem Cell, Transplantation, Treatment, Transcription Factor.

INTRODUCTION

Advancements in the understanding of spinal cord injury (SCI) and repair have taken great leaps in the past decade. The greatest challenge in SCI research has been the translation of what is known at the basic science level, and applying it to the clinical setting. Although, the understanding of the molecular basis of SCI has become more cogent, the therapeutic options continue to be limited. Although neural stem cells (NSCs) have been studied for over a decade, they continue to be at a translational research stage. Initially, exogenous stem cells (ExSCs) were an exciting breakthrough. However due to various reasons discussed below their clinical application as independent interventions has started to diminish. Recently, endogenous stem cells (eNSCs) have become more of an interest in SCI. Nature's endogenous process of healing spinal cord injuries was once thought to be counterproductive. However, as discussed below there has been evidence to suggest on the contrary. In sum, the function of eNSCs has been encouraging, and could help intervene in altering this natural process of healing. Further, its translation into clinical practice could improve the quality of life for numerous patients affected by SCIs.

SPINAL CORD INJURY

Epidemiology

Epidemiologically, SCI has been and continues to be a huge burden to society. Some reports find the incidence to be as low as 8 and as high as 246 cases per million depending on political, geographical, sociological and economic differences [1 - 4]. The mean age has increased from 28.3 years in the 1970s to 37.1 years surveyed in 2005-2008. Most concerning has been the epidemiological age group affected. As the majority affected have been in their peak productive years of their lives [5]. Also, more than 50% of SCIs affect the cervical region of the spinal cord, which is more likely to cause total body paralysis [6 - 8].

Mechanism of SCIs were either traumatic or nontraumatic [3]. Traumatic causes in young adults (age 15-29 years) were motor vehicle accidents (50%), violence (12%) and sport-related injuries (10%) [7, 9, 10]. Traumatic causes in the elderly (age >65 years) were mainly accidental falls [8 - 12]. Non-traumatic causes in

both age groups were congenital and inflammatory spinal cord disorders, tumor compression, vascular ischemia vertebral spondylosis [13].

Pathophysiology

Primary Phase

SCI has been categorized into primary and secondary phases [14, 15]. The primary phase occurs due to compression and contusive injury resulting in shearing, laceration and stretching of the spinal cord. This exerts forces causing disruption of axonal function [16, 17]. It was suggested that at this stage full transection of the spinal cord was rare. There was sparing of demyelinated axons found at the subpial rim [17 - 21]. Animal models suggest that a minimum of 10% preservation of original axons could lead to significant neurological recovery [22, 23].

Secondary Phase

The pathophysiology of the secondary phase injury was directly proportional to the injuries occurring in the primary injury phase [17]. This phase was marked by a negative environment for neuronal regeneration [17]. The pathophysiology was marked by various processes such as ischemia, excitotoxicity, vascular dysfunction, oxidative stress and inflammation resulting in cell death [18, 24, 25]. Secondary phase injury was deleterious to surviving surrounding neurons, which further impaired functional recovery [14, 26]. The secondary phase had sub-phases which were: immediate (<2 hours), acute (2 hours – 2 days), subacute (3 days to 2 weeks), intermediate (2-3 weeks – 6 months) and chronic (>6 months) [17, 18, 27, 28].

Immediate phase consisted of upregulation of tumor necrosis factor (TNF)-alpha, interleukin (IL)-beta and elevation of extracellular glutamate [29 - 32]. This resulted in necrosis of neurons secondary to ischemia, lipid peroxidation, hemorrhage, reactive oxygen species (ROS) production, edema and cell membrane disruption. This lead to early loss in function and neurogenic shock [16, 17, 23, 33, 34].

A patient with SCI has shown to be initially clinically intervened in the acute phase. This phase was further divided into early acute and subacute stages [17]. Genes that change after injury have been classified as either “early” or “late” genes. They were responsible for activating cascades at various stages [35]. After an SCI event there was an upregulation of pro-apoptotic, cell cycle and oxidative stress mediating genes. Moreover, there was downregulation of antiapoptotic genes, genes involved in neural excitation, neurotransmission, electrochemical

CHAPTER 2

Toward Induced Pluripotent Stem Cells for Clinical Use: Sources, Methods and Selection

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Abstract: Since their development by Yamanaka in 2007, much progress has been made in the last decade toward the use of human induced pluripotent stem cells (iPSCs) in clinical practice. In this review, we will focus on the various sources of somatic cells for human iPSC generation, the methods used for generating human iPSCs, their characterization, and the progress on directed differentiation toward several cell types. We will also describe current efforts to prevent culture-driven mutations and the selection of nontumorigenic cells for clinical use. A comprehensive comparison of such methods will aid in the establishment of standardized techniques and highlight areas in which further research is still needed.

Keywords: cMyc, Cell Reprogramming, Cell Therapy, Clinical Grade IPSC, Differentiation, Disease Modelling, Epigenetics, Episomal Vectors, Embryonic Stem Cells, Genetic Stability, Induced Pluripotent Stem Cells, Klf4, Matrigel, Oct4, PBMCs, Pluripotency, Quality Control, Retrovirus, Sox2, SSEA, Transfection, Transplantation.

INTRODUCTION TO INDUCED PLURIPOTENT STEM CELLS

Cell reprogramming has historically been achieved by various methods, such as somatic cell nuclear transfer [1, 2], cell fusion with pluripotent cells [3], incubation with pluripotent cell extracts [4], and derivation of pluripotent embryonic germ cells (EGCs) from primordial germ cells by addition of a specific cocktail of growth factors [5, 6].

More recently, studies on the genes involved in stemness led to the ground-breaking work of Yamanaka and Thomson, showing that forced expression of just

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four transcription factors can reprogram mouse and human fibroblasts into a full pluripotent state, the so-called induced pluripotent stem cells (iPSCs) [7 - 9].

Because the use of human embryonic stem cells in the clinic is controversial due to the ethics involved, to be able to reprogram adult somatic cells into pluripotent stem cells, thus avoiding any ethical or moral deterrent, was remarkable; so much so that Yamanaka received the Nobel Prize in Medicine only 6 years after the initial discovery.

SOURCES

The selection of the cell source for reprograming is a very important aspect to take into account, given not every cell type is equally efficiently reprogramed or equally accessible. Here, we will take a look at the beginnings of iPSC derivation and the various cell types that have since been successfully used to create iPSCs. We will also evaluate the various cell types that are actually being used in research, particularly with prospective clinical applications in mind.

In the Beginning: Mouse Embryonic Fibroblasts

Although in 2006 Takahashi and Yamanaka were not the first to achieve or prove cellular reprograming of somatic cells [7], remarkably, they identified a combination of only four transcription factors (Oct4, Sox2, Klf4, and cMyc, named Yamanaka factors or OSKM), present in embryonic stem cells that could completely reverse somatic differentiated cells into an embryonic pluripotent state [7]. The first generation of iPSCs that Yamanaka's group obtained failed to produce viable chimeras when injected in mouse embryos. Upon changing the selection method for the reprogrammed cells, a collaborative effort with other laboratories allowed Yamanaka and colleagues to develop the second generation of iPSCs, which could successfully form chimeras and therefore give rise to the three germ layers of the embryo. The actual establishment of the first iPSC lines was performed on mouse embryonic fibroblasts (MEFs) and later on adult mouse tail tissue fibroblasts (TTFs). Once 24 genes that were deemed important transcripts for the maintenance of embryonic stem cell (ESC) properties were identified, they transduced various combinations of those genes by retroviral transfection in MEFs, and the resulting cells were screened for pluripotency [7]. As previously mentioned, the exact cocktail of transcription factors needed for the reprograming event was found: *Oct3/4*, *Sox2*, *c-Myc* and *Klf4*. When transfected with these four genes, their activity was reactivated and the whole transcription program of the cell changed to that of the pluripotent state in both MEFs and TTFs.

Today, the reprograming of mouse fibroblasts is not the focus of new research on

iPSCs, given their clinical application potential is limited. However, mouse fibroblast-derived iPSCs are still used in other types of experiments, for example to test new methods for increased efficiency or to establish new reprograming pathways that differ between cell lineages. MEFs are also widely used as feeder cells in iPSC cultures, although they need to be treated beforehand with mitomycin or irradiation to prevent growth.

Human Fetal and Neonatal Cells

Human Neonatal Fibroblasts

Human neonatal fibroblasts were used as a source for iPSC generation soon after the discovery that younger cells are more easily reprogrammed [10, 11]. These fibroblasts are usually extracted from foreskin samples rather than skin biopsies, which is a safer approach for young individuals. They have the same pros and cons as regular fibroblasts: they require a somewhat long culture time for expansion purposes but then are more easily reprogrammed than other cell types (see below), especially taking into account the young "age" of the cells. They have also been proven to be efficiently reprogrammed even after long-term cryopreservation, which is important in terms of the availability of the samples [12].

Fetal Stem Cells

Fetal stem cells have also been used extensively as a source for reprogrammed cells. Because they are already stem cells, they are easier to reprogram, have better tissue repair capacity, and sometimes have more rapid growth kinetics than adult stem cells. They can be obtained from newborn and fetal tissues, such as blood, liver, and bone marrow, and also from extraembryonic structures, such as the placenta, cord blood, and amniotic fluid [13, 14].

Fetal stem cells are different from adult stem cells in the sense that they are half way between a pluripotent and a stem cell-like phenotype, presenting markers of both states [14]. However, the initial pool of cells can be very heterogeneous, thus affecting the efficiency of the reprogramming between experiments and making it more difficult to pinpoint the exact nature of the original colony-forming cell [15].

Human Umbilical Cord Endothelial Cells

Human umbilical cord endothelial cells (HUVECs) present a very accessible source of good quality cells for reprogramming, given the extraction procedure carries no risks whatsoever. Also, these neonatal cells are young and therefore less prone to carrying somatic mutations at the time of isolation. HUVECs can also be

CHAPTER 3

Reprogramming of Adipose-Derived Stem Cells to Neuronal-Lineage Cells is Regulated by Both Cell Signalling and Redox Status

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Abstract: The generation of specific neuronal-lineages for cellular therapies hold great promise for nervous system disorders. Stem cells can offer regenerative and replacement therapies of the nervous system through cell signalling, which is directed by the addition of cytokines and neuronal growth factors. Adipose-derived Stem Cells (ASCs) are capable of differentiating into neuronal and glial cells through induced cell signalling pathways and altered redox status. In this chapter, we addressed the dynamic changes within ASCs in response to the changes in its *milieu* - a pre-requisite for trans-differentiation *in vitro*. We considered the functional use of ASCs as a regenerative tool in recovering neuronal cells by focusing on ligand expression and their effects on transmembrane receptors. We also discussed various levels of cell signalling capable of modifying epigenetic programming for trans-differentiation processes. Finally, we underlined the fact that harnessing of Reactive Oxygen Species (ROS) and ROS-mediated cellular signalling is a secret recipe for successful differentiation of stem cells *in vitro*.

Keywords: Adipose stem cells, Cell signalling, Differentiation, Epigenetics, Growth factors, Glia, Laser, Light, Neurons, Photobiomodulation, Reactive Oxygen Species.

INTRODUCTION

The most common types of neurological disorders include *spinal cord injuries* caused by accidents, and *stroke* caused by cerebral haemorrhages. These pathologies result from neuronal malfunction or cellular damage that triggers a cascade of signalling events leading to paralysis and cognitive dysfunction. None of the methodologies devised for neuronal differentiation is 'wholly successful'. There is also a need to cogitate on the mechanisms leading to neuroglial transfor-

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mation. Currently there is no research indicating an effective biological or chemical differentiation inducer for Adipose-derived Stem Cells (ASCs), nor the mechanism behind driving ASCs to neurons or glia. Our understanding about various ligands driving the differentiation of ASCs to neurons is very scarce. Researchers have adopted different differentiation approaches or mix-matched them based on literature, where their attempts ended up in 'neuron-like' bodies. These undifferentiated ASCs are electrophysiologically inactive and not useful for targeted therapy. This chapter attempted to connect neuroglial differentiation with relevant and recent literature pertaining to cellular changes, whilst focussing on cell signalling which is often controlled by the redox status.

NERVOUS SYSTEM

Development and Distinction of Neuronal-Lineage Cells

Pre-natal development of the central nervous system (CNS) is characterized by the formation of neural plates that give rise to the neural tubes. A highly integrated and spatially distinct neural stem or progenitor population of neuroepithelial progenitor cells (NEPs) exists within these structures. Initially, NEPs undergo symmetric division to expand, but later switch to asymmetric division for giving rise to lineage-restricted progenitors. Neural Precursor Cells (NPCs) is a mixed population of Neural Stem Cells (NSCs) and neural progenitor cells, which give rise to neurons and supporting cells [1]. The last stage of CNS development is marked by dendrite branching, axonal elongation as well as formation of synaptic junctions to facilitate impulse transmission. In the developing nervous system, NSCs undergo asymmetric division to produce glial-restricted progenitors, which generate astrocytes, oligodendrocytes and Schwann cells [2]. Finally, NSCs are also the source of specific- neurotransmitter release cholinergic, dopaminergic and GABAergic neurons. Table **1** indicates the functional as well as anatomical significance of different glial cell types configuring the human CNS.

Anatomy of Central and Peripheral Nervous Systems

The nervous system is broadly divided into two principal parts—CNS and the peripheral nervous system (PNS). The CNS consists of the brain and the spinal cord, while the PNS consists of sensory and motor nerves that interconnect with the periphery. The latter act as a communication system between the periphery of the body and the spinal cord. The CNS decipher sensory input from the PNS and translate it into an appropriate motor response. The nervous system is also classified according to its control over the body functions. The somatic nervous system (SNS) is responsible for all the sensory or cognitive activities followed by the movement of skeletal and ocular muscles. The autonomic nervous system

(ANS) controls all involuntary activities concerning cardiac and smooth muscles as well as endocrine glands.

Table 1. Glial cell types supporting the central and peripheral nervous systems.

Cell types	Function
Neuroglia (glia)	helper cells of neurons for anatomical and functional support
Schwann Cells	glial cells that produce myelin sheaths around axons of the PNS
Astrocytes	glial cells that function in the BBB in the piamater of the CNS
Oligodendrocytes	glial cells that produce myelin sheaths on axons of the CNS (white matter)
Satellite Cells	glial cells that are helper cells to sensory and autonomic nerves of the PNS
Ependymal Cells	glial cells that are part of the choroid plexus and produce CSF
Microglia	glial cells that are phagocytic immune cells that find & destroy invaders

NOTE: Schwann Cells, Satellite Cells, Oligodendrocytes, Microglia, Astrocytes, and Ependymal Cells are the six types of neuroglia (glia) cells that support neuronal growth and activity. *Abbreviations: CNS, Central Nervous System; PNS, Peripheral Nervous System; CSF, Cerebrospinal Fluid.*

The nervous tissue consists of neuronal and glial cells, which are the functional and supportive cells of both the CNS and PNS, respectively. Morphologically, neurons have a soma or cell body upon which several extensions emerge and interconnect with the neighbouring cell. These processes, referred as dendrites are the structures that receive sensory input from the surroundings. Each neuron will have at least one of these processes as a prominent extension, referred to as the axon. They are the functional units of neurons that are responsible for the transmission of impulses to neighbouring cells or neurons. It is important to note that a bundle of axons in the CNS is mentioned as tract, while the same in the PNS is a nerve. Similarly, a localized collection of neuron cell bodies is referred to as a nucleus in the CNS and as a ganglion in the PNS.

Diseases and Disorders of the Nervous System

Neurodegeneration is a condition characterized by progressive deterioration of neuronal morphology and functional characteristics due to ischaemia or haemorrhage, deposition of intracellular toxic proteins or accumulation of genetic mutations [3]. Alzheimer's disease and amyotrophic lateral sclerosis is characterized by neurovascular dysfunction resulting in the impairment of the blood–brain barrier function. This evidently result in a lack of nutrition to cholinergic nerve cells and the impairment of clearing neurotoxic molecules resulting in neurodegeneration [3]. Another well-studied condition, Parkinson's disease, is caused by the degeneration of dopaminergic neurons resulting in a gradual dysfunction of the motor neuronal system. Any hypofunction of N-methyl-D-aspartic acid-type glutamate (NMDA) receptors at the cerebral cortical

CHAPTER 4

Advances in Skin Regeneration and Reconstruction

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Abstract: The skin represents the largest and most accessible organ of the body, and it is subjected to numerous aggressions such as infections, physical wounds and diseases. After a moderate-intensity injury, the intrinsic regenerative mechanisms of the skin lead to restoration of the tissue integrity. However, in some pathological cases such as chronic wounds and extensive burns, the healing capacity of the tissue is overwhelmed. In order to resolve these injuries, innovative therapies based on miRNAs as well as paracrine and trophic activities of stem cells combined with biomaterials are currently being developed. This chapter begins with a description of skin biology, followed by the main stages of wound healing including the key cells and molecules involved. Next, we describe the most studied miRNAs relevant for chronic wounds therapies and the proposed methods of delivery. Regarding cellular therapy, the main adult differentiated cells as well as stem cells available from different sources, are presented. Then, we address the commercially available skin substitutes and also the latest innovative approaches, including 3D bioprinting, for combining biomaterials with the activity of the cells previously described, in order to promote wound repair and regeneration. This chapter concludes with current challenges and future perspectives regarding the use of stem cells for skin regeneration.

Keywords: Biomaterials, Cellular therapy, Chronic wounds, microRNAs, Skin, Skin biology, Stem cells.

INTRODUCTION

The skin is the largest organ of the human body. Situated at the interface between the organism and the exterior environment, it carries out the role of mechanical and waterproof barrier. As a consequence of its position and function, it is often injured as a result of the action of various external factors (mechanical, physical or chemical). Although, in normal physiological conditions, the process of wound healing is extremely effective, resulting in a nearly perfect tissue repair, especially at young ages, there are circumstances in which the repair mechanisms are damaged. The result is either a chronic wound (ulcerative skin damage) or a scar

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tissue (hypertrophic or keloid). As a consequence of the prolonged requirement of medical care and elevated rate of reoccurrence, chronic wounds represent a significant burden to the healthcare resources all over the world [1]. Furthermore, chronic wounds represent an important problem of the modern society, as they are a significant cause of morbidity, leading to a poor quality of life, disability, pain, depression and social isolation [2]. This type of skin injury is associated with an intensified inflammatory process, which can be a result of mechanical stimuli (pressure ulcers) or a consequence of other severe dysfunctions, among which diabetes is particularly prevalent in our days.

Burns represent another major type of skin injury, which require special treatment and often result in debilitating scars. The healing of a burn depends on the depth of the wound. The deep second-degree and third-degree burns, where the reticular dermis is affected, result in hypertrophic scars and contraction [3]. Moreover, in severe burns, even though they involve a single organ, almost all systems of the body are influenced, so that it becomes a generalized disorder [4]. Furthermore, sepsis and the accompanying invasive infection are still the primary cause for death after the first 24 hours, with a peak at two weeks after the accident.

Last, but not least, the skin can be affected by genetic diseases. Given the fact that the skin comprises various cell types expressing specific molecules with role in maintaining the structural integrity of the skin, even a single mutation in one of these molecules can disrupt the skin structure, leading to cell separation, blistering, and eventually, wounds [5]. For example, epidermolysis bullosa comprises a group of inherited, disfiguring and painful diseases, which determines skin fragility causing blisters in response to even minor trauma [6].

Next, we will begin the with a short description of the skin structure and function, after which the main stages of the wound healing process and the main small non-coding RNAs (microRNAs) will be described. Given the fact that the standard wound care is not effective in severe skin injuries/diseases, we will focus our attention on potential innovative therapeutic approaches such as cellular therapy and the use of available skin substitutes as wells as new strategies to improve them.

SKIN BIOLOGY

The skin has many vital functions, by being an immunologically active sensory and excretory organ and also, by preventing loss of excess water and electrolytes, while having an important role in thermoregulation [7, 8].

The integumentary system is formed by the skin and its derivative structures: sweat glands, sebaceous glands and mammary glands, hair, hair follicles, and

nails [9]. The skin is organized in three layers: the stratified cellular epidermis, the underlying dermis, and subcutaneous tissue [10]. The epidermis is a continually renewing stratified epithelium of ectodermal origin, composed of 80% by keratinocytes, which are cells specialized in the synthesis of keratin a fibrous threadlike structural protein with a protective role [2]. These cells are disposed in five distinct layers: stratum basale (basal cell layer), stratum spinosum, stratum granulosum, stratum corneum and stratum lucidum- this last one being present in skin exposed to friction, such as skin on the feet or hands (Fig. **1**). Besides keratinocytes, several other cell populations exist in the epidermis: melanocytes, which have the ability to produce melanin and to donate this pigment to the keratinocytes [11], Langerhans' cells, which have immunological functions, and Merkel cells with tactile functions [10].

The **stratum basale (SB or basal layer**) represents a single layer of mitotically active cells which replaces the cell loss on the outer epidermis [12]. The process of cell kinetics in the epidermis is complex and is represented by the balance between growth with differentiation and cell death [10]. In epidermis, the turnover takes between 40-56 days and plays an important role in maintenance of the skin barrier function [13]. Thus, in the basal layer, there are cells that can persist undifferentiated and, in this way, the self-renewing of the basal cell population is preserved. These cells with unlimited capacity for self-renewal and the ability to generate daughter cells that undergo terminal differentiation are named stem cells. They are situated in small clusters in the basal layer of the epidermis. The basal keratinocytes are characterized by the expression of specific keratins: K5 and K14, and contain gap junctions for cell communication, desmosomes for cell-to-cell attachment, hemidesmosomes for connecting with the basement membrane, and an extracellular matrix [14, 15]. The basal layer synthesizes, secrets, and assembles an extracellular matrix (ECM), which ensure the stability of the connections and communication between the epidermis and the dermis [16]. Immediately above the basal cell layer, keratinocytes become more irregular in shape and form the **stratum spinosum (SS or squamous layer)**. This layer consists of 8 to 10 sheets of keratinocytes which express K1 and K10 and have a reduced potential for cell division. In the middle of this layer, the Langerhans cells are located. The intercellular spaces between squamous cells are bridged by abundant desmosomes which promote mechanical coupling between cells of the epidermis and aid in flexibility, enabling the epidermis to better withstand the effects of friction and abrasion [9] (Fig. **1**).

The **stratum granulosum (SG or granular layer)** is composed of two or three layers of flatten cells containing basophilic keratohyalin granules consisting of profilaggrin, protein that is eventually cleaved into filaggrin peptides [8].

CHAPTER 5

MiRNA Mediated Stem Cell Therapy for Cardiac Arrhythmia

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Abstract: Arrhythmias are electrical disturbances resulting in irregular heartbeats. Multiple ion channels orchestrate the coordinated propagation of electric stimuli within the heart. Dysfunction of the ion channels, which may result from genetic alterations in ion channel genes or their aberrant expression, can render electrical disturbances predisposing to cardiac arrhythmias. MicroRNA (miRNA) is a type of small RNA belonging to non-protein coding RNAs and is involved in RNA interference, thereby functioning as a regulator of gene expression. Recently, the role of miRNA in cardiac conduction diseases, arrhythmogenesis and their therapeutic potential has been implicated. Stem cell therapies for cardiac arrhythmia have attempted to modulate defective ionic currents by delivering engineered cells. The modulation or engineering of stem cells with appropriate miRNAs could improvise stem cell-based therapies for arrhythmia. This chapter would review the role of miRNAs in cardiac rhythmic disorders and its potential in diagnosis and treatment of cardiac arrhythmia.

Keywords: Arrhythmia, Channelopathy, Conduction Disorder, Epigenetic Regulation, Ion Channels, MicroRNA, Regenerative Medicine, Stem Cells, Stem Cell Therapy, SCD, Target Prediction.

INTRODUCTION

Cardiac arrhythmia is a worldwide serious problem responsible for high rate of cardiovascular mortality and morbidity. An arrhythmia is a condition where the heart beat becomes irregular. Cardiac arrhythmias are significantly associated with increased risks of cardiovascular complications and sudden death [1]. Arrhythmias affect people worldwide, atrial fibrillation alone is estimated to affect 33.5 million people [2]. Rhythmic disturbances of cardiac function are underlying symptoms of a wide range of diseases, disorders or side effects of

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drugs, which could result in either tachycardia or bradycardia. Arrhythmia are classified as atrial tachycardia, sinus bradycardia or ventricular arrhythmia depending on the origin of the rhythm disturbance. It also may be further classified on the basis of the rate and mechanism (triggered, ectopic, reentry).

Arrhythmias could manifest themselves as ones with mild to serious effects with some being fatal enough to result in sudden cardiac death. Ventricular arrhythmias are the most important cause of sudden cardiac death (SCD). Occurrence of SCD is estimated to be 4 to 5 million cases per year worldwide [3, 4], thereby highlighting arrhythmias and SCD as the most serious cardiovascular diseases worldwide. Arrhythmias do not manifest at any specific age and could be acquired or inherited. In addition to genetics, the occurrence of arrhythmia is also influenced by other factors like smoking, caffeine, drugs, ischemia, infarction, *etc.* Acquired arrhythmias are a manifestation of diverse abnormalities (electrical, structural, metabolic, neurohormonal, or molecular alterations) in multiple pathological conditions (heart failure, hypertension, diabetes mellitus, hyperthyroidism, aging, *etc.*) [5].

The rhythm of the heart beat is controlled by the electrical conduction system (CS) of the heart. The conduction system is responsible for the maintenance of normal blood pressure and ensures proper blood circulation. The electrical activity of the heart is mediated by ion channels and transporters. Ion channels, which are integral membrane protein complexes, facilitate a selective passage of ions across the cell membrane through various gating mechanisms including voltage, pH, ligands *etc.*, thereby being pivotal in the generation of electrical impulses in excitable cells. The coordinated activity of these ion channels is instrumental in the generation and propagation of the cardiac action potential as well as normal ECG pattern Fig. (**1**). The unique signature pattern of ion channels across different regions of the heart is responsible for the unique action potentials generated by them. The normal cardiac rhythm is regulated by the precise action potential in each type of cell in the various compartments of the heart. The electrocardiogram (ECG) is a result of the cumulative effect of the cellular action potential generated by the meticulously timed action of different ion channels. Dysregulation or functional impairment of ion channels, transporters, intracellular Ca^{2+}-handling proteins *etc.* can result in the generation of structural and metabolic substrates, which have the potential to culminate in electrical disturbances predisposing the individual to conduction abnormalities and arrhythmias [6].

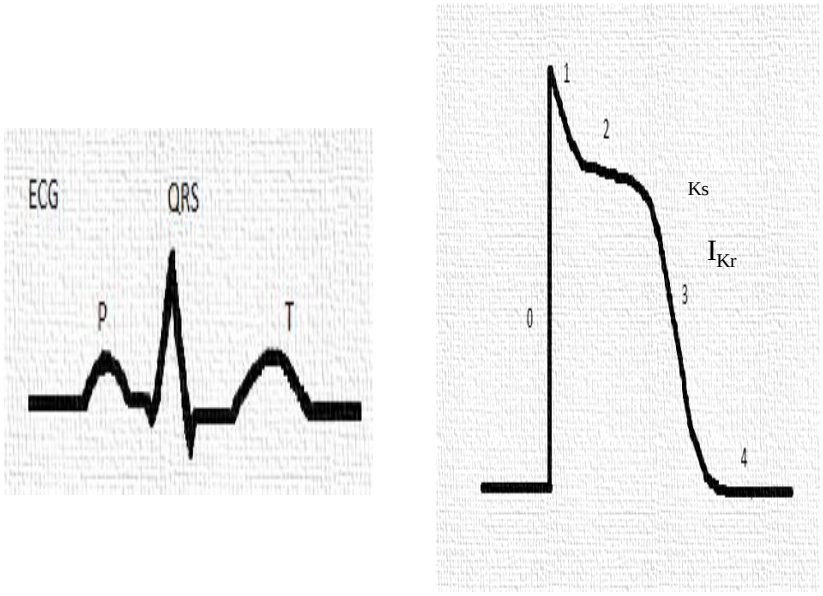

Fig. (1). The normal ECG pattern and the ionic currents involved in myocardial action potential.

CARDIAC CHANNELOPATHIES AND ARRHYTHMIA

Electrocardiographic abnormalities are the result of underlying changes in the cellular action potentials which arise due to aberrant cell-to-cell coupling, diseased conditions of the heart, congenital ion channel abnormalities, drug intervention, or electrolyte imbalance [7 - 9]. Primary electrical diseases of the heart refer to rare inherited cardiac arrhythmias in the absence of structural abnormalities of the heart that are associated with mutations in ion channel genes. Wilde and Bezzina (2005) have summarized the ion channel genes that are associated with these inherited arrhythmia conditions [10]. These findings were based on studies of various mutants and highlighting the important contribution of ion channels in maintenance of normal cardiac rhythm. The term cardiac channelopathies is used to refer to ion channel disorders resulting in anomalies of cardiac function, including but not limited to arrhythmias. Molecular studies revealed mutations in genes coding for specific ion channels being responsible for heritable arrhythmogenic disorders such as LQTS, SQTS, Brugada syndrome and idiopathic atrial fibrillation. The findings of genetic basis of the inherited arrhythmia syndromes promoted better understanding of the molecular genetics of cardiac arrhythmias and the role of different ion channels. The development of molecular diagnostic tests has also increased the chances of an early detection of the subjects at high risk of developing arrhythmias.

CHAPTER 6

Malaria Treatment and Stem Cells

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Abstract: In some countries, Malaria is still a challenge. The highest rates of mortality are reported in sub-Saharan Africa, where children under five years of age, pregnant women and immunocompromised patients are the most vulnerable groups. People living in these endemic areas still do not receive proper antimalarial therapy. Insecticides resistance, antimalarial drug resistance and commercialization of counterfeit and substandard antimalarials, are key factors contributing to complexity in malaria control; trying to find new alternatives to treat and control malaria, some members of the scientific community, have recently started to work in the field of stem cell therapy in experimental malaria models. The purpose of the present chapter is to make a general review concerning various aspects of the use of stem cell therapy and how these findings could improve clinical aspects during malaria pathogenesis and could be used in the field of antimalarial drug design. An overview of the effect of the parasite on the stem cells production in the host, as in hematopoiesis and in neurogenesis, is also described.

Keywords: Adult Neurogenesis, Bone Marrow Stromal Cells, Cognitive Dysfunction, Hepatocytes, Hematopoiesis, Hippocampus, Malaria, Malaria Pathogenesis, Malaria Drug Design, Mesenchymal Cells, Memory, Murine Malaria, Neurogenesis, *Plasmodium falciparum*, *Plasmodium vivax*, Stem Cell Therapy.

INTRODUCTION

Malaria is a disease of great global impact due to the number of deaths it produces annually. The World Health Organization (WHO) in 2015 reported 214 million cases world wide and 438,000 deaths. The most serious situation is in the countries of sub-Saharan Africa, where children under the age of five are parti-

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cularly exposed and it is one of the main causes of infant mortality in that region [1].

Due to the limitations in the adoption and implementation of chemoprophylaxis worldwide, in 2014, 306,000 children under the age of five died of malaria and the percentage of pregnant women receiving preventive therapy was less than 20% and only half of the population in endemic areas slept under mosquito nets treated with insecticides [2].

Human malaria is attributed to four species of *Plasmodium*: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, which have a particular geographic distribution. *P. falciparum* is distributed in all malaria areas of the world but predominates in sub-Saharan Africa, *P. malariae* has a similar distribution to that of *P. falciparum* but is much less frequent, *P. vivax* predominates in Central America and India, and *P. ovale* is found in Africa and is very rare outside of it [2]. Malaria is transmitted to the human by an Anopheles female mosquito that injects the parasites into the host blood stream while feeding.

Plasmodium vivax predominates in countries with the lowest incidence of malaria, accounting for more than 70% of cases in countries with less than 5000 cases reported each year. According to the WHO, 2015, the malaria caused by *P. vivax* is concentrated in all Latin America, Oceania and Southeast Asia. In all endemic regions of vivax malaria, severe cases and deaths have been reported. Due to the difficulty in controlling *P. vivax*, its incidence has decreased more slowly than that of *P. falciparum* in places where the two species coexist. *P. vivax* can then persist as the main cause of malaria and constitute the main challenge for its elimination [3].

Malaria life cycle can be observed in Fig. (**1**).

More than 50 years ago, it was reported that under laboratory conditions, some ape *Plasmodium* parasites were transmitted to humans [4]; it is now known that *Plasmodium knowlesi* is emerging as a very important zoonotic pathogen, which is being exported from endemic areas to other parts of the world through travelers; the infection can be potentially spread through blood transfusions, bone marrow transplantation and congenital infections [5].

Knowledge about the pathogenesis of the parasite that produces malaria has been obtained mostly from non-human animal models such as murine models [6]. The pathogenesis of malaria is complex and is the result of the destruction of the infected erythrocytes and the host's immune-response to the infection. Only the blood stage of the parasite is involved in the pathogenesis of the disease [7].

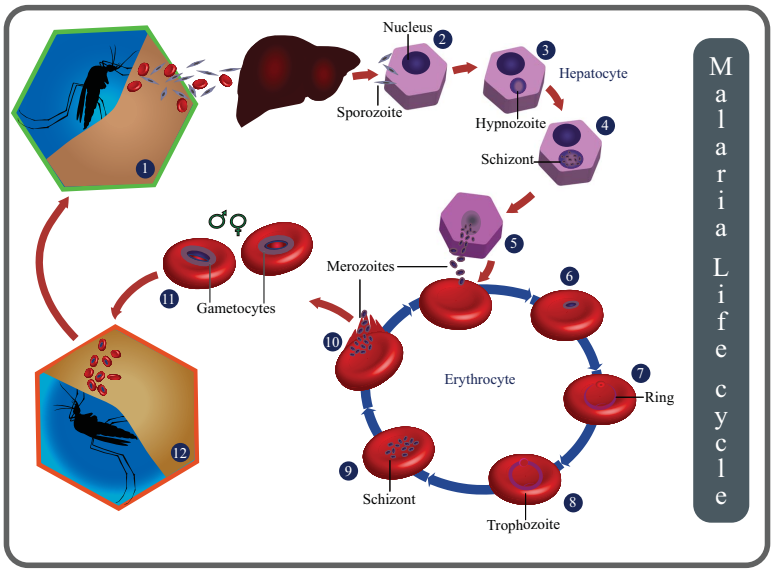

Fig. (1). Malaria life cycle. 1. Female Anopheles mosquito injects sporozoites into the host blood stream while feeding. The parasites replicate in the hepatocytes developing schizonts that liberates thousands of merozoites (2-5). *Plasmodium vivax* remains as hypnozoite inside the liver cells (3). Free merozoites invade the red blood cells and start the intraerythrocytic cycle (6-10). In the last schizogony, gametocytes (11) are formed and ingested by a female *Anopheles* which will develop new infective sporozoites (12).

Mice blood smears with different stages of *Plasmodium yoelii yoelii* can be observed in Fig. (**2**).

In malaria, different pathogenic mechanisms can be observed: block of microcirculation at different organs by sequestration of infected erythrocytes to the endothelium, exacerbated production of proinflammatory cytokines and oxidative stress. The products derived from the parasite during the intra-erythrocytic cycle induce the production of proinflammatory mediators such as TNF, IL-1, IL-6 and IFN-γ. These mediators induce the production of other cytokines and enzymes that perpetuate the inflammatory cascade. Serum levels of proinflammatory cytokines are increased in patients with complicated malaria or cerebral malaria [8, 9].

In malaria, oxidative stress occurs with increased generation of reactive oxygen species, such as superoxide anion, hydrogen peroxide, hydroxyl radical and peroxynitrite, which are produced by the host's inflammatory cells to control parasitemia, but are also produced directly by the intra-erythrocytic parasite, when in the digestive vacuole it transforms the hemoglobin into methemoglobin. The production of reactive oxygen species in malaria has been demonstrated by

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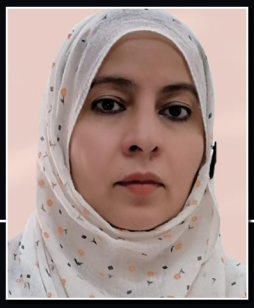
ATTA-UR-RAHMAN, *FRS*

Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/ Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.



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As recognition of her contributions to science, she has been awarded with 03 International awards like Fellowship from Islamic World Academy of Sciences, Postdoctoral fellowship from Ministry of Culture and Education, Spain and a Young Chemist Award from Third World Academy of Sciences, Italy. She also has several national awards on her credit.