Preface

This book presents current research in the study of new technologies, developments and applications of microRNA and non-coding RNA. Topics include the role of microRNA and short interfering RNAs in plants; microRNAs, major affective disorders and suicidal behavior; microRNA in endoparasites; the functional roles of non-coding RNAs in glioma and their clinical implications; identifying miRNA function in innate immunity; and how multiple IsomiRs and diversity of miRNA sequences unveil evolutionary roles and functional relationships across animals.

Chapter 1 - In plants, two main categories of small RNAs (sRNAs) are distinguished based on their biogenesis and function: microRNAs (miRNAs) and short interfering RNAs (siRNAs). MiRNAs control the expression of cognate target genes by binding to reverse complementary sequences, resulting in cleavage or translational inhibition of the target RNAs. To date, nearly one thousand miRNA genes have been identified from various plant species as shown in miRBase and published reports, and this number is ever increasing. siRNAs have similar structure, function and biogenesis as miRNAs but are derived from long double-stranded RNAs and can often direct DNA methylation at target sequences. Plant miRNAs play important roles in maintenance of genome integrity and organ development such as leaf morphogenesis, floral organ identity and vascular development. The plant miRNAs also function in feedback regulation in the sRNA pathway and in directing biogenesis of some siRNAs. In this review, characteristics and functions, as well as signaling of miRNAs and siRNAs in plant have been summarized, with emphasis on their roles in plant developmental regulation of plants. Further studies on miRNAs and siRNAs are needed as they will not only provide us with important clues to understanding regulation mechanisms among miRNAs, siRNAs and their target genes, but also push forward future developments in this exiting field.

Chapter 2 - Major affective disorders are common and disabling conditions associated with significant psychosocial impairment and suicidal risk in the general population. At least 3–4% of all depressive individuals die by suicide. At a molecular level, affective disorders and suicidal behaviour have been recently associated with abnormalities in structural and synaptic plasticity. A recent hypothesis suggested that small non-coding RNAs (ncRNAs), in particular microRNAs (miRNAs), play a critical role in the translational regulation at the synapse. Here the authors summarized the current literature on miRNAs putative subcellular localization and sites of action in mature neurons analyzing their role in neurogenesis, synaptic plasticity, pathological stress changes, major affective disorders and suicidal

behaviour. miRNAs have played a fundamental role in the evolution of brain functions. The perturbation of some intracellular mechanisms as well as impaired assembly, localization, and translational regulation of specific RNA binding proteins may affect learning and memory, presumably contributing to the pathogenesis of major affective disorders and perhaps suicidal behaviour. Also, miRNA dys-regulation has also been associated with several neuropsychiatric diseases. However, further evidence are needed in order to directly clarify the role of miRNAs in major affective disorders and suicidal behaviour [Abstract words (189); keywords (4): microRNAs; synaptic plasticity; major affective disorders; suicidal behaviour].

Chapter 3 - MicroRNAs (miRNAs) are short, non-coding RNAs that down-regulate gene expression post-transcriptionally by binding to the mRNA of their target genes. The importance of miRNAs in key biological processes such as development, cell proliferation, cell differentiation and metabolism has been widely documented since their discovery. To date, more than 25,000 mature miRNAs have been reported in 193 species of animals and plants (miRBase, release 19, http://www.mirbase.org/index.shtml). Parasites face different environments during their complex life cycles and their ability to respond to environmental and developmental signals may require miRNA-mediated networking and fine-tuning of gene expression. Some protozoan and helminth parasites have shown peculiarities in their small-RNA machinery. For example, the small RNA-generating machinery of the apicomplexan parasite *Toxoplasma gondii* is phylogenetically and functionally related to that of plants and fungi. Among protozoan parasites, miRNAs have been reported in *Giardia lamblia*, *Trichomonas vaginalis*, *Trichomonas foetus*, *Pentatrichomonas hominis, Trypanosoma brucei*, *Entameoba histolytica*, *Neospora caninum* and *Toxoplasma gondii*. Most of the protozoan miRNAs do not share significant homology to any of the known miRNAs of plants and metazoans. Some interesting roles such as silencing of variant surface proteins have been proposed for a number of miRNAs in *G. lamblia*. However, in other unicellular parasites such as *Trypanosoma cruzi* and *Plasmodium falciparum* no miRNAs or small RNA machinery components have been identified. Among helminth parasites, miRNA identification and expression profile analyses have been performed by a combination of bioinformatic and experimental approaches. So far, miRNAs have been reported in the nematodes *Angiostrongylus cantonensis*, *Brugia pahangi*, *Brugia malayi*, *Haemonchus contortus*, *Ascaris suum* and *Trichinella spiralis;* the trematodes *Fasciola hepatica, Fasciola gigantica*, *Schistosoma mansoni*, *Schistosoma japonicum*, *Clonorchis sinensis and Orientobilharzia turkestanicum* and the cestodes *Echinococcus granulosus, Echinococcus multilocularis* and *Taenia saginata*. Conserved as well as novel miRNAs have been reported, some of them are abundantly expressed and display developmental regulation, suggesting important roles in several helminth parasite species. miRNA targets have been bioinformatically predicted in parasitic protozoa and helminths but only a small number of protozoan miRNAs has been experimentally validated. Unraveling miRNA roles in parasite biology and host interaction remains a challenge. For this end, bioinformatic strategies adapted to each phylum together with experimental strategies such as miRNA silencing and/or overexpression should be set up. The knowledge of miRNA roles in parasite development and survival in their hosts will in turn provide new control and intervention strategies against the worldwide distributed and mostly neglected diseases they produce.

Chapter 4 - Glioma is the commonest form of primary brain tumors with poor clinical outcome. MicroRNAs (miRNAs) are endogenous non-coding RNAs (ncRNAs) of

approximately 22 nucleotides (nt) that regulate the expression of target messenger RNAs (mRNAs) at the post-transcriptional level. Increasing evidence shows that miRNAs play key roles in the biological behavior of glioma. They are involved in a wide variety of gliomagenic processes including cell proliferation, invasion and apoptosis by acting as tumor suppressors or oncogenes. MiRNAs may potentially be exploited as biomarkers for diagnosis, prognostication, and even therapeutic targets. Recently, the characterization of long non-coding RNAs (lncRNAs) has received considerable attention. Like their smaller non-coding miRNA counterparts, lncRNAs represent a novel class of gene regulators in cancer. Early evidence demonstrates that the differential expression of specific lncRNAs may also serve as novel biomarkers for diagnosis and therapies. In this chapter, the authors summarize the recent advances in the investigations of miRNAs and lncRNAs in glioma, and discuss their potentials for clinical use.

Chapter 5 - miRNA regulates immunity by modulating the expression of key genes involved in leukocyte development and function. Furthermore, immunity includes innate and adaptive systems comprising myeloid-derived cells and lymphocytes which mediate nonspecific and pathogen-specific responses. Innate immunity encompasses the generation of myeloid-derived cells and their ability to mount a rapid and appropriate response to pathogen-associated danger. Here the authros highlight common components of research investigating the role of miRNA in innate immunity. The identification of miRNAs involved in immunity generally begins with the profiling of samples generated ex vivo or in vitro which include the cell types and immunological conditions of interest. From here a core set of methods and technologies are utilised. Various technologies exist for quantifying miRNA expression, including qRT-PCR and microarray platforms capable of profiling all known human miRNAs as listed by miRBASE. miRNA array expression data may be confirmed by qRT-PCR before in silico analysis of predicted miRNA targets, and these miRNA-target interactions validated by dual luciferase assays. Finally, the biological significance of any miRNA is dependent upon the degree of modulation it exerts combined with the importance of it target within the relevant system. A widely used approach for ascertaining this is to perform experiments in which the miRNA is constitutively expressed or knocked-down and altered function or experimental outcome tested. To date these methods have revealed several important roles for miRNA in the functioning of the immune system. As the technologies and techniques described here continue to develop so will our understanding of the function of miRNA within immunity and with our prospects of manipulating expression for therapeutic gain.

Chapter 6 - As crucial negative regulatory molecules, small non-coding RNAs, microRNAs (miRNAs), have been widely studied, especially for their functions and evolutions. Multiple miRNA variants, termed as isomiRs, are recently detected and identified from a given miRNA locus due to alterative and imprecise cleavage of Drosha and Dicer. Compared to the canonical miRNA sequence, these isomiRs may be found various 5' and/or 3' ends (various 3' ends are more popular), 3' additional non-template nucleotides, 5' and seed modification events. Similarly, miRNA sequences in different animal species also show diversity as well as species specificity. The miRNA sequences in different animal species may be involved in different length distributions, 5' and/or 3' ends, transition/transversion and insertion/deletion. From a given miRNA locus, internal multiple isomiRs and various miRNA sequences across species are quite similar and have close relationships. Indeed, they also implicate potential functional and evolutionary relationships. By applying high-throughput sequencing technique, multiple isomiRs from a given miRNA locus can be

identified with different sequence counts, length distributions and various expression patterns, although isomiR repertoire and their expression patterns always are stable across different tissues, samples and even animal species. Most isomiRs were 3' isomiRs which had the same seed sequences, while 5' isomiRs were found to have new seed sequences via the phenomenon of "seed shifting". Many miRNAs were detected a set of isomiRs, in which 1-3 were abundant isomiRs characterized by a narrow length distribution (20-23 nt) due to dominant cleavage. Indeed, specific miRNA sequences have been identified in specific animal species through classical experimental validation. The annotated miRNA sequence is always derived from the most abundantly expressed isomiR, which is prone to be detected by traditional detection. Therefore, the authors speculate that the most dominant isomiR is not randomly selected, but is strictly regulated by complex regulatory network from selection and evolutionary pressures. Various miRNA sequences can unveil dynamic evolutionary processes across different animal species (cross-species) and across different miRNAs (within species), especially simultaneously involved in the diversity of pre-miRNA and pri-miRNA sequences. Furthermore, more miRNA* sequences, ever as passenger strands of miRNA sequences, are identified as active and functional miRNA sequences. These potential functional miRNA* sequences and potential more functional isomiRs (such as 5' isomiRs with novel seed sequences by changing 5' ends) will complicate miRNA-mRNA and miRNA-miRNA interaction, and enrich coding-non-coding RNA regulatory network.