List of Figures

Figure.1.1. Retinoblastoma Fundus photograph ................................................................. 3
Figure.1.2. Retinoblastoma Eye ball and sections. .............................................................. 3
Figure.1.3. Differences in the allelic changes for Genetic and sporadic forms of Retinoblastoma. ......................................................................................................................... 5
Figure.1.4. Loss of heterozygosity in Retinoblastoma: Image represents chromosome 13q homologs and Rb1 shown with two markers A/b and a/B ........................................... 13
Figure.1.5. MicroRNA biogenesis pathway. ........................................................................... 14
Figure.1.6. Y-79 and treated Y-79 extracted total RNA was resolved on Bioanalyser chip. ........................................................................................................................................ 30
Figure.1.7. EpCAM is expressed in RB tumors: (A) EpCAM mRNA ranges from 2-100 fold in RB tumors (n=30). ............................................................................................................. 36
Figure.1.8.1. Effect of EpCAM gene knockdown on miRNA expression profile in Y79 cells (a part of heat map) ........................................................................................................... 37
Figure.1.8.2. Quantitative Real Time PCR validation confirmed expression of miR-130b and miR-181c in RB tumors and microarray results in EpCAM silenced Y79 cells ........................................................................................................................................ 39
Figure.1.9.1. Antagomir treatment in Y79 and WERI-Rb-1 cell line lead to suppression of miR-130b and miR-181c ................................................................................................. 40
Figure.1.9.2. Inhibition of miR-181c and miR-130b decreased cell viability and invasion in Y79 and WERI-Rb-1 cells. .................................................................................................. 42
Figure.1.9.3. Representative images of invasion assay. .......................................................... 43
Figure.1.9.4. Chromosomal locations of significant down regulated miRNAs upon EpCAM silencing in Y79 cells. ........................................................................................................... 46
Figure.2.1. Retinoblastoma MicroRNA profile ...................................................................... 69
Figure.2.2.A-E: Graph shows quantitative Real-Time PCR analysis of miR-17, miR-18a, miR-19b-1, miR-20a and miR-92a-1 in control (n=20) and RB serum samples(n=20). ....................................................................................................................... 71
Figure.3.1. RB serum vs Tumor miRNA ............................................................................... 80
Figure.3.2. Flow chart of Bioinformatic analysis for RB microRNA target prediction . 82
Figure 3.3. RB microRNA gene ontology ................................................................. 83
Figure 3.4. Retinoblastoma negative regulatory network genes............................... 86
Figure 3.5. Retinoblastoma positive regulatory network genes............................... 87
Figure 4.1. PDMS general device model ................................................................. 95
Figure 4.2. Schematic representation of LNA hybridization with miRNA ............... 97
Figure 4.3. PDMS mask ....................................................................................... 100
Figure 4.4. Coating layers on PDMS surface (PAV method) ................................. 101
Figure 4.5. Coating layers on PDMS surface (APTES method) ............................. 102
Figure 4.6. MicroRNA bioassay with sensor miR-18a probe ................................. 104
Figure 4.7. MicroRNA bioassay with sensor miR-18a probe—Spectroscopic method 106
Figure 4.8. Schematic representation of different layers coated on PDMS ............ 108
Figure 4.9. FTIR analysis of PAV method ............................................................. 110
Figure 4.10.1. Schematic representation of different layers coated on PDMS ......... 111
Figure 4.10.2. FTIR analysis of APTES method ...................................................... 113
Figure 4.10.3. PDMS coatings ellipsometry ........................................................... 114
Figure 4.11.1. Hybridization on PDMS channels .................................................. 115
Figure 4.11.2. PMMA design outlay ...................................................................... 121
Figure 4.11.3. Dye mixing experiment conducted at IIT Chennai ....................... 122
Figure 4.11.4. Dye mixing experiment conducted at Achira Labs, Bangalore ...... 123
Figure 4.11.5. MicroRNA Hybridization in PMMA channels ............................... 124
Figure 4.11.6. MicroRNA Hybridization in PMMA channels—Microscopic spectral data-1 ............................................................................................................. 124
Figure 4.11.7. MicroRNA Hybridization in PMMA channels—Microscopic spectral data-2 ............................................................................................................. 125
Figure 5.1. Methylation PCR analysis of APC-2 in RB tumours and Y79 cells ....... 137
Figure 5.2. Sequencing analysis of APC-2 methylation specific PCR product ...... 138
Figure 5.3. Sequencing analysis of Bisulfite converted APC-2 PCR product ......... 138
Figure 5.4. Immunofluorescence study on 5’-AZC treated Y79 cells ................. 139
Figure 5.5. Flow cytometry analysis of APC-2 expression on 5’-AZC treated Y79 cells ............................................................................................................... 140
Figure 5.6. Western blot analysis of APC-2 in Y79 cells ....................................... 140
Figure 5.7. Co-localization analysis on APC-2 and β-catenin. 142
Figure 5.8. Image indicates the MIOM1 cells co-localization experiment. 143
Figure 5.9. EpCAM to APC-2 RB regulation. 144