Abstract of the thesis

Epithelial ovarian cancer (EOC) is one of the most lethal gynecological malignancies; this lethality is attributed to late diagnosis, rapid advancement and heterogeneous nature of tumor, which imparts chemoresistance and aggressive post therapeutic recurrence. Understanding of molecular tumor behavior that influences gene and protein expression and their effects on cellular functions during EOC progression could pave the way for improvement in disease management. Hence, the present study was undertaken to –

(a) identify aberrant epigenetic changes that alter the transcriptional status of target genes during EOC progression, and

(b) explore the possibility of applying these changes in predictive assessment of epigenetic drugs.

Whole genome promoter DNA methylation patterns of an in vitro progression model of EOC [a pair of cell lines earlier established in the lab that included immortal, pre-transformed cells (A4P), and transformed cells derived from A4P (A4T)] were analyzed for identification of differentially regulated genes through DNA and histone methylation. Promoter hypomethylation emerged as a distinctive feature of EOC progression contrary to hypermethylation in other cancers. Correlations between the methylome and transcriptome assigned functional relevance to differential methylation during progression. Further comparison with the methylomes and transcriptomes of grade-wise segregated serous ovarian adenocarcinoma samples available with TCGA (The Cancer Genome Atlas) provided a clinical relevance to the study. Overlapping the A4 and TCGA datasets thus identified a common association of 5 hypo- and 3 hyper-methylated genes with pre-transformed / early grade, and 15 hypo - and 2 hyper-methylated genes with transformation and malignant high-grade disease. Bisulfite genomic sequencing and quantitative PCR of 6 genes confirmed CYC1, POGK, MAL, and MEST as hypomethylated and upregulated, while FBN1 and
PTGIS were suggested to be hypermethylated and downregulated during progression.

Genome-wide histone methylation profiles (H3K4me3, H3K9me3 and H3K27me3) during A4 progression established through ChIP-on-chip (CoC) were analyzed to identify gene promoters differentially enriched with histone marks. To establish the independent effect of altered histone modifications on the gene expressions, 23 genes that were exclusively enriched with histone but not DNA methylation were studied in detail. 14 of these followed the histone code (H3K4me3 - upregulated, H3K9me3 and H3K27me3 - downregulated expression); this correlation was further validated through ChIP-qPCR and semi quantitative PCR.

Expression modulation of the twenty epigenetically regulated genes (6 differential promoter and 14 histone methylation), following in vitro epigenetic drug treatment(s) identified PTGIS, MEST and RXRγ as potential predictive biomarker for 5-Aza-dC/TSA, Curcumin and CBB1007 respectively. Intra tumor heterogeneity (ITH; molecular, morphological and functional heterogeneity within tumor) can be better understand with evolutionary concept of tumor, where due to selection pressure during tumor development and progression favorable accumulated genetic and epigenetic alterations could be considered as principal reason for ITH. This concept also suggests that modulation of heterogeneity before and after treatment may best uncover the genetic / epigenetic changes that lead to therapeutic resistance and relapse. These were further evaluated for epigenetic drug treatments in NOD/SCID mice on a background of differing tumor cell responses arising from ITH that considers different populations based on the proliferative hierarchy, genetic instability and different cell cycle stages. 5-Aza-dC effectively stabilized cell cycling, restricted genetic instability, and de-repressed PTGIS expression, while TSA led to emergence of drug-resistant progenitors lacking PTGIS expression. Profiling MEST and RXRγ for Curcumin and CBB1007 respectively indicated an inability of Curcumin and CBB1007 in restricting residual tumor regenerative capabilities.
Conclusively, our study provides novel insights into epigenetic regulation in EOC progression and identifies potential biomarkers for evaluating efficacy of epigenetic drugs. Our study further indicates that each epigenetic drug targets / enriches different set of cell populations as identified from differential modulation of biomarker expression in residual tumour cells after treatment. Such approaches may assign a new functional interpretation of drug efficacy and cell tumor responses in ovarian cancer.