# Chapter VII

## Conclusion and Future perspectives

## 7.1 Conclusion

This study makes an effort to unveil the transcriptomic profile of GBC and distinctly identified key genes and pathways driving the development of GBC. For the first time, this study has generated a transcriptomic dataset on two groups of GBC patients – GBC without gallstones (GBC) and GBC with gallstones (GBC+GS) from Assam, India. The findings revealed distinct differential gene expression patterns and biological pathways between these GBC groups. The hub DEGs in GBC were primarily associated with cell adhesion processes, while those in GBC+GS were linked to the cell cycle and signal transduction pathway. *LMOD1* and *SMAD4* emerged as crucial genes in the GBC and GBC+GS groups, respectively, exhibiting significant downregulation and acting as potential tumor suppressors. Therefore, *LMOD1* and *SMAD4* can act as potential biomarkers for GBC and GBC+GS patients.

Another significant finding of this study is the identification of specific novel lncRNAs in the GBC and GBC+GS groups. This study identified crucial regulatory lncRNAs and miRNA targets using integrated network-based approaches. The results demonstrate that the DE lncRNAs and DEnlncRNAs were markedly downregulated in both GBC groups. The ceRNA regulatory network analysis identified potential novel lncRNAs. These lncRNAs interacted with DEmRNAs that are significantly involved in oncogenic signal transduction and cell cycle signaling pathways. Given the well-established links between dysregulated signal transduction and cell cycle signaling in cancer, targeting these lncRNAs holds promise for improving the prognosis and management of GBC patients. The identification of crucial novel lncRNAs and their targets have advanced our molecular comprehension of lncRNA involvement in GBC pathogenesis, offering promising avenues for further investigation and functional validation.

The study was further directed toward identifying regulatory transcription factors in GBC pathogenesis and development. Transcriptional regulatory network analysis revealed the association of a unique set of transcription factors between the two GBC groups. The TNF-alpha signaling pathway and the HSV1 infection were identified to be the common pathological pathways associated with the DETFs. The hub DETFs in the GBC group are found to be linked with EMT, whereas, the hub DETFs identified in the GBC+GS group are significantly associated with the inflammatory response pathway. KLF15 and MECOM were identified as crucial DETFs in the GBC and GBC+GS groups respectively. These TFs were found to interact

with both DEmRNAs and DElncRNAs, which indicate that KLF15 and MECOM play an important regulatory role in the development and pathogenesis of GBC.

**Summary of the key findings of the thesis:**

- Integrative data analysis of publicly available GBC transcriptome datasets revealed that the DEGs exhibit significant downregulation and show unique gene expression patterns compared to other cancers of the hepatobiliary tract. The identified DEGs in GBC are significantly associated with signal transduction pathways that regulate the cell cycle system, cell-cell adhesion processes, and apoptosis.

- The potential candidate DEGs identified in GBC compared to gallstones in each follow-up period (stages) were associated with distinct biological processes and signaling pathways- ECM interaction processes (follow-up period ≤ 3), MAPK signaling ((follow-up period between 5-10 years) and TCR and PI3K signaling pathways (follow-up period > 10 years) that play important roles in cancer development, This implies that the progression of gallstones to GBC occurs through the dysregulations of multiple signal transduction pathways at different stages (initiation-progression-metastasis) with distinct pathological spectrum.

- For the first time this study has generated transcriptome datasets from the clinical tissue samples from two different groups of GBC patients – GS-independent gallstones and GS-associated gallstones cases from the most high GBC-risk region (Assam, India). The transcriptome analysis revealed distinct differential gene expression patterns and biological pathways between these GBC groups.

- The key potential DEmRNAs identified in GBC were primarily associated with cell adhesion processes, while those in GBC+GS were linked to the cell cycle and signal transduction pathway. *LMOD1* and *SMAD4* identified as potential DEGs in the GBC and GBC+GS groups, respectively. These DEGs shows marked downregulation as compared to control group and therefore, *LMOD1* and *SMAD4* can serve as potential diagnostics/targeted biomarkers for GBC and GBC+GS patients.

- One of the most important findings of the thesis is the identification of novel lncRNAs specific to GBC and GBC+GS. *LINC00852*, *MSTRG.53675.1* in GBC+GS and *DIO3OS*,

and *MSTRG.16633.1* in GBC groups were identified as potential regulatory lncRNAs. In the GBC+GS group, *LINC00852* and *MSTRG.53675.1* lncRNAs were found to interact with potential hub DEmRNAs involved in FoxO signaling pathways which induce pro-tumorigenic effects by modulating crucial regulatory pathways and promote tumorigenesis in gallbladder with gallstones. However, in the GBC group, the lncRNAs identified indirectly interact with the cell-adhesion molecules and contribute to tumor invasion through EMT and tumor-microenvironment interaction, which ultimately leads to increased cell proliferation and metastatic gallbladder carcinogenesis.

- Transcriptional regulatory network analysis revealed the association of a unique set of transcription factors between the two GBC groups. The TNF-alpha signaling pathway and the HSV1 infection were identified to be the common pathological pathways associated with the DETFs in both the GBC groups. The hub DETFs in the GBC group are found to be linked with cell migration and EMT processes, whereas, the hub DETFs identified in the GBC+GS group are significantly associated with the immune response related pathway. This is the first study that reports the association of *KLF15* and *MECOM* in GBC and GBC+GS pathogenesis respectively. These TFs were found to interact with both DEmRNAs and DElncRNAs, which indicates that *KLF15* and *MECOM* acts as master regulators in the pathogenesis of GBC.

The work carried out in this thesis presents a unique and high-quality transcriptome dataset involving two groups of GBC patients. The research offers new perspectives on the systemic aspects of GBC pathogenesis and has uncovered important molecular signatures, including mRNA, lncRNA, and TFs along with pathways linked to GBC development. The identified findings have the potential to be translated into clinical settings, indicating promising applications for future medical practices.

## 7.2 Future perspectives

The research work carried out in this thesis has further possibilities to be worked on, which comprises of:

1. The genes identified in this study can be further screened in a large cohort for the development of diagnostic or prognostic biomarkers specific to GBC patients. This could aid in early diagnosis and a better prognosis.

2. Transcriptomic studies of GBC patients from other parts of India can be performed to understand the gene expression variation in terms of lifestyle and food habits. Such studies might unravel genetic diversity, environmental influences, and disease heterogeneity, and will have potential implications for developing precision medicine.

3. Functional characterization of the novel lncRNAs identified in GBC could be performed to understand their biological functions and mechanistic role in GBC pathogenesis and progression.