Non-coding RNAs as emerging molecular targets in gallbladder cancer

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Abstract
Gallbladder cancer is one of the most common cancers of biliary tract with aggressive pathophysiology, now emerging as a global health issue. Although minority of gallbladder patients could receive such curative resection due to late diagnosis, this increases the survival rate. Lack of potential target molecule(s) for early diagnosis, better prognosis and effective therapy (of gallbladder cancer) has triggered investigators to look for novel technological or high throughput approaches to identify potential biomarker for gallbladder cancer. Intervention of non-coding RNAs in gallbladder cancer has been revealed recently. Non-coding RNAs are now widely implicated in cancer. Recent reports have revealed association of non-coding RNAs (microRNA or miRNA and long non-coding RNAs or lncRNAs) with gallbladder. Here, we present an updated overview on the biogenesis, mechanism of action and role of non-coding RNAs in gallbladder tumorigenesis, their prognostic & therapeutic potentials (efficacies) and future significance in developing effective biomarker(s), in future, for gallbladder.

Abbreviations
1. GBC  Gallbladder cancer
2. lncRNA  Long non-coding RNA
3. miRNA  microRNA
4. LincRNA  Long intergenic non-coding RNAs
5. MALAT1  Metastasis-associated lung adeno carcinoma transcript 1
6. CCAT1  Colon cancer associated transcript 1
7. ITGB1  Integrin beta 1
8. UCRs  Ultraconserved regions
9. RISC  RNA Induced Silencing Complex
10. SNP  Single nucleotide polymorphism
11. HMGA2  High mobility group AT hook 2

Key words: Biomarker, Gallbladder Cancer, Long non-coding RNAs, MicroRNA
Introduction

The first report of gallbladder cancer came back in 1777 (de Stoll 1777). It is one of the most common cancers of biliary tract and is now becoming a major global health issue, particularly for middle aged women (Barbhuiya et al., 2009; Randi et al., 2008; Siegel et al., 2015). Late symptomatic onset and diagnosis at advanced stage increases difficulties in prognosis and therapy. Curative resection is the most common practice to treat gallbladder cancer patients. However, only minority of gallbladder cancer patients get the benefits of such curative resection. Increasing the survival rate of gallbladder cancer patients has remained a major challenge at present. This can only be achieved once appropriate and specific diagnostic, prognostic or therapeutic biomarker(s) is/are identified and developed to check the tumorigenic pathophysiology, i.e., inhibition of tumor birth and growth. Recent advancements in high throughput technologies have contributed significantly to these goals.

Non-coding RNAs are a class of RNAs transcribed consistently, covering more than 75% of the genome (Eddy 2001; Djebali et al., 2012). ncRNAs are a large and heterogeneous class of RNAs, which include small nucleolar RNAs (snoRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), small nuclear RNAs (snRNAs), piwi interacting RNA (piRNA), long non-coding RNAs (lncRNAs), long intergenic non-coding RNAs (lincRNAs) and ultraconserved regions (UCRs). LncRNAs share the largest portion of the mammalian non-coding transcriptome (Mercer et al., 2009). They are of size longer than 200 nt and are processed from the unprocessed transcripts (Guttman et al., 2009; Ulitsky and Bartel 2013). LncRNAs and miRNAs are well known for diverse functions. Their implication in cancer is yet not fully elucidated, however, they are being considered as the major target for biomarker discovery. In
this review, we have discussed biogenesis, mechanisms of action and disruptions of these non-coding RNAs in gallbladder cancer. A brief account of future prospect of biomarker development for early diagnosis, better prognosis and effective therapy of gallbladder cancer is also given.

**Biogenesis and functions**

**miRNAs:** The biogenesis of miRNAs has been widely reviewed (Bartel 2004; Esteller 2011). Generally, miRNAs are embedded in the genomic regions or within the exons of protein coding genes (Kim and Kim 2007). In brief, miRNAs are initially transcribed inside the nucleus by RNA polymerase II as a long, capped (at 5’ end) and polyadenylated (at 3’ end) long primary transcript, with hairpin stem loop structure, known as poly-cistronic miRNA clusters or pri-miRNAs. These miRNAs are recognized and further trimmed by an RNA polymerase III enzyme, Drosha, with DGCR8 as a co-factor, to ~70 nt long sequence, known as pre-miRNAs. These molecules are now exported to cytoplasm by a cargo, Exportin-5/Ran-GTP. Further trimming is done by another RNA pol III enzyme, Dicer, producing ~22 nt long mature miRNA. Helicase separates the strands of the double stranded miRNA, producing single stranded stable miRNA, while, the other strand is processed for autolytic degradation. Dicer-TARBP2 (TARR RNA Binding Protein 2) load the stable mature miRNA strand to RNA Induced Silencing Complex (RISC) to mechanistically target 3’-Untranslated Regions (3’ UTRs) of protein coding mRNAs, consequently acting in post transcriptional regulation via two mechanism: mRNA degradation for the perfect complementarity and inhibition of translational initiation for incomplete complementarity (He and Hannon 2004). Loading of miRNAs to RISC and regulatory mechanism of RISC are tightly regulated (Krol et al., 2010). Recent reports have
claimed 3’-UTRs and 5’ UTRs as emerging targets of miRNAs (Esteller 2011). Argonaute proteins play crucial role in assembling RISC components, where they act as catalytic endonuclease. *lin-4* is the first miRNA discovered by Ambros lab and is found to have base sequence complementary to 3’-UTR of *lin 14* in *C elegans*. It triggers the transition of cell division from the first larval stage to the second larval stage (Lee et al., 1993). Recent reports have identified the functional roles of miRNAs in many biological and cellular processes, including cell proliferation, differentiation, apoptosis, senescence and development (Esteller 2011).

**Long non-coding RNAs:** LncRNAs are a class of non-protein coding RNAs. These diverse and heterogeneous class of RNAs are defined by size discrimination of more than 200 nt long transcripts without open reading frame. Generally, LncRNAs are also transcribed by RNA polymerase II. These long LncRNA transcripts are subjected to normal pre-splicing editing, such as 5’ capping and 3’ polyadenylation (Li and Chen 2013). There are few reports suggesting the role of LncRNAs in transcriptional regulation of genes. LncRNAs function in the epigenetic modification of DNA, specific to chromatin remodeling structures at specific loci (Navarro et al., 2006). Hundreds of LncRNAs have been identified at the human HOX gene loci. They are expressed during transcription and regulate the chromatin structure, which involves histone modification enzymes and RNA polymerase (Rinn et al., 2007). LncRNAs are also involved in X-chromosome inactivation in mammals. During X-chromosome inactivation, polycomb complex is recruited by X-inactivation Specific Transcript (*XIST*), a LncRNA, to silence the X-chromosome in-cis (Plath et al., 2003). Interestingly, a recent study reported interacting network of LncRNAs with protein coding genes and even with miRNAs (Ma et al., 2015).
Deregulation of miRNAs in gallbladder cancer

**MicroRNAs:** With the discovery of miRNAs (Lee et al., 1993) and RNA interference (Fire et al., 1998), the scope of research to identify novel potential biomarker has widened to a great extent. This has provided a novel strategy for investigators to discover specific early diagnostic biomarker of gallbladder cancer. Efforts are still ongoing to elucidate significant association of miRNAs with gallbladder cancer. Disruptions in the nucleotide sequences have also been detected. Variations in the single nucleotide polymorphism (SNP) of pre- & pri-miRNAs may alter the expression level of the respective miRNAs, thereby, suggesting possibility to identify potential biomarkers of gallbladder cancer. Genetic variants of miR-196a, miR-499, miR-146a, miR-27a, miR-570 and miR-181a are not associated with gallbladder cancer (Srivastava et al., 2010; Gupta et al., 2015). However, accumulation of these three variants (miR-27a, miR-570 and miR-181a) was found to influence the chemo-therapy toxicity, i.e., poor prognosis in gallbladder cancer (Gupta et al., 2015). The miRNA profiling in BK5.erbB2 gallbladder cancer mice revealed deregulation of few miRNAs. When treated with histone deacetylase inhibitor, PCI-24781, gallbladder cancer cells showed downregulation of miR-21, miR-142-3p, miR-142-5p and miR-223, whereas, miR-122, upregulated (Kitamura et al., 2012). The level of miR-146b-5p was observed to be very low in tumor tissues of gallbladder (Cai et al., 2015). Restoring miR-146b-5p expression in the gallbladder cancer cell line, SGC-996, inhibited tumor growth through enhanced apoptosis and G1 phase arrest. miR-146b-5p also regulates the expression of EGFR (Cai et al., 2015) (see figure 1 and table 1). This small sized miRNA has potential to inhibit tumor growth and arrest defective cell cycle steps. Elevated levels of miR-20a and TGF-β1 lower the survival rate of gallbladder cancer patients. Exogenous introduction of miR-20a in vivo and in vitro enhanced epithelial to mesenchymal transition (EMT) and metastasis of gallbladder cancer.
cancer cells via targeting 3’UTR of Smad7 and nuclear translocation of β-catenin (Chang et al., 2013). Down-regulation of miR-34a and long telomere length increased the poor prognosis and survival of gallbladder cancer. Introduction of miR-34a in CD44+CD133+ gallbladder cancer tumor stem-like cells inhibited cell proliferation in vitro and xenograft tumor growth in vivo [Jin et al., 2014]. MiR-133a, miR-133b, miR-143-3p, miR-145-5p and miR-1 are significantly downregulated in gallbladder cancer (Letelier et al., 2014). Ectopic expression of miR-1 and miR-145-5p in gallbladder cancer cell line, NOZ, inhibited cell viability and colony formation, and decreased the expression of VEGF-A and AXL (Letelier et al., 2014). Downregulation of miR-138 and upregulation of Bag-1 were also observed in gallbladder tumor tissues. Ectopic expression of miR-138 results in the silencing of Bag-1 and proliferation of gallbladder cancer cells (Ma et al., 2015). The proposal for potential use of miR-155 as a therapeutic biomarker came from a report in which gallbladder cancer patients with upregulated miR-155 showed poor prognosis with lymph node metastasis as compared to those with down-regulated miR-155. This may also be associated with cell proliferation, aggressive behavior with increased lymph node metastasis and vessel invasion (Kono et al., 2013). It is suggested that xanthogranulomatous cholecystitis can be distinguished from gallbladder cancer based on expression profile of miR-155. Consistency of gallbladder cancer specific miR-155 expression without any other alteration during inflammatory conditions, associated with pancreatobiliary malfunction and possibility of its accurate detection in serum/bile, mark it as a prospective novel diagnostic marker for gallbladder cancer (Kono et al., 2013). A recent study has added miR-187, miR-143 and miR-202 to a list of key molecules having clinicopathological significance in gallbladder cancer (Li and Pu 2015). These small RNAs could be used as potential non-invasive markers for early diagnosis. Qiu et al., (2014) observed remarkably high level of miR-182 in metastatic gallbladder
cancer tissues. TGF-β also induces expression of miR-182 and metastasis of gallbladder cancer cells (Qiu et al., 2014). Cell adhesion molecule 1 (CADM1) is a predicted target of miR-182, and miR-155 suppresses CADM1 level enhancing cell invasion. Blockage of miR-182 increases the level of CADM1 in gallbladder cancer cells (Qiu et al., 2014). Absence of miR-182 blocked metastasis of gallbladder cancer cells to far off tissues, e.g., lungs. This is another supporting evidence for consideration of miR-182 as potential therapeutic target via inhibition of tumor cell metastasis (Qiu et al., 2014). Gallstone disease is generally considered as an early pathological condition of gallbladder cancer and also a well-known risk factor (Qiu et al., 2014). Whole genome sequencing of gallstone disease tissues identified six differentially expressed miRNAs, where the levels of miR-133a and miR-891a were found significantly decreased, while that of miR-210, miR-200c, miR-194 and miR-192 were enhanced (Yang B et al., 2015). ATP11A (ATPase, class VI, type 11A) is a transporter gene across membranes and is also essential for generation and maintenance of phospholipid asymmetry in lipid bilayer (Graham 2004; Pomorski et al., 2004). Elevated level of miR-210-3p decreases the level of its target gene, ATP11A (ATPase, class VI, type 11A) in GSD tissues. miR-210 is associated with hypoxia, which affects the cellular processes, such as metabolism, survival, proliferation, migration and angiogenesis (Semenza 2001). High level of miR-210 enhances progression of cholangiocarcinoma cells in vivo in Balb/c mice with chronic cholestasis (an important pathogenic factor for gallstones) via HIF-2a-miR-210 pathway (Yang B et al., 2015). The level of miR-26a is significantly low in gallbladder cancer (Zhou et al., 2014). The high mobility group AT hook 2 (HMGA2) is a target of miR-26a, with inhibitory functions in cell cycle (Li et al., 2006). Ectopic expression of miR-26a efficiently blocks cell proliferation and cell cycle transition (G1/S) (Zhou et al., 2014). Recently, it was suggested that downregulation of miR-135
and subsequent increase in the level of its target gene, very low density lipoprotein receptor (VLDLR), in gallbladder cancer, may prove useful in the process of biomarker development (Zhou et al., 2015). Restoration of miR-135 expression disturbs cell proliferation and cell cycle of gallbladder cancer cells via p38 MAPK pathway (Zhou et al., 2015). These studies draw attention to focus our investigation on the utility of miRNAs, based on their role, as potential biomarkers for early diagnosis, better prognosis and effective therapy. Very interestingly, reports of cross kingdom interconnection of plant MIR-168a with human/mouse low-density lipoprotein receptor adapter protein 1 (LDLRAP1) (Zhang et al., 2012), therapeutic effects of Olea europaea leaf extract with miR-181b, miR-153, miR-145, miR-137 and let-7d in glioblastoma multiforme (GBM) cells (Tunca et al., 2012) and curcumin (diferuloylmethane) to miR-22 and miR-199a in pancreatic cancer cells (Sun et al., 2008), have opened up new strategies to dissect out possible far reaching therapeutic implications. Similar investigation in gallbladder cancer may provide greater insight into our understanding not only on the mechanism, but also in therapy, in future.
Long non-coding RNA:

There is increasing interest in dissecting out the role of lncRNAs in gallbladder cancer. The role of lncRNAs in gallbladder cancer is very limited. *Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)*, an oncogenic lncRNA, is known for its tumor associated functions. *MALAT1* is upregulated in gallbladder cancer tissues (Wu et al., 2014). *MALAT1* also plays a regulatory role in pre-mRNA alternate splicing mechanism by modulating the levels of active serine/arginine splicing factors (Hutchinson et al., 2007; Tripathi et al., 2010). The processing
mechanism is altered by the absence of MALAT1, thereby, enhancing the tumorigenic activity. MALAT1 interacts with unmethylated form of CBX4, which controls relocation of genes between polycomb complex and interchromatin bodies (Yang et al., 2011). Targeting the interacting molecules and, or subsequent introduction of inhibitory drugs or molecules will help dissect out the molecular pathway with respect to the aforesaid expression state or role. Silencing of MALAT1 lowered cell proliferation of gallbladder cancer, but, increased G2/M cell cycle transition. When anti-tumor effect of MALAT1 was blocked in vivo by injecting MALAT1-depleted or control SGC-966 cells into the left axilla of nude mice, growth of tumors or xenografts was found significantly inhibited. Knockdown of MALAT1 reduced the migration or invasion of gallbladder cancer cells. Matrix metalloproteinase 9 (MMP9), an enzyme involved in the breakdown of extracellular matrix during metastasis, is a downstream target of MALAT1. Loss of MALAT1 expression reduces the expression of MMP9, thereby, activating ERK/MAPK pathway (Wu et al., 2014). This study supports the evidence that MALAT1 is a potential therapeutic and prognostic marker of gallbladder cancer. CCAT1 (Colon cancer-associated transcript-1), another lncRNA, was first identified in colon cancer (Nissan et al., 2012). CCAT1 is found to promote tumor development. Interestingly, upregulation of CCAT1 is consistent with the down regulation of miR-218-5p in gallbladder cancer tissues (Ma et al., 2015). The binding and regulation of miR-218-5p by CCAT1 in gallbladder cancer tempts further to find out more number of miRNAs targeted by lncRNAs (see figure 2A and table 2). Such interactions are common to other cancers also (Wang et al., 2009; Han et al., 2013). Thus, overexpression of CCAT1 enhances the expression of Bmi1, through miR-218-5p mediated regulation of Bmi1 in gallbladder cancer cells (Ma et al., 2015). Over expression of Integrin beta-1 (ITGB1), an lincRNA, in gallbladder cancer cells enhances cancer cell proliferations, cell migration and
metastasis (Wang et al., 2015). In a study to test the therapeutic potential of ITGB1 using lentivirus mediated transfection of ITGB1, it was found that knockdown of ITGB1 inhibited cancer cell proliferation, cell migration and metastasis of gallbladder cancer cells (Wang et al., 2015), recommending its potential therapeutic utility in future. HOTAIR (HOX antisense intergenic RNA) is a direct target of c-Myc gene via interaction with upstream promoter region of HOTAIR. HOTAIR is induced by c-Myc in gallbladder cancer cells (Ma et al., 2014). Ectopic expression of c-Myc increases the level of HOTAIR. HOTAIR and miR-130a are found to be self-regulated via a repression loop. Knockdown of HOTAIR inhibited proliferation and metastasis of gallbladder cancer cells. Introduction of miR-130 inhibitor can restore the functional state earlier blocked by the knockdown of HOTAIR (Ma et al., 2014) (see figure 2B). Cellular level changes in gallbladder cancer by lncRNA are shown in figure 3. Similarly, upregulation of KIAA0125 enhances proliferation and migration of metastatic gallbladder cancer cells via induction of Vimentin, and suppression of β-catenin (Lv et al., 2015). Vimentin is a member of intermediate filament family, and plays key role to maintain cellular integrity. Cell migration and invasion is strongly inhibited by suppressing EMT activity when KIAA0125 knocked down gallbladder cancer cells are used. Thus, KIAA0125 is suggested to be a potential therapeutic target in gallbladder cancer cases.
Running title: **Non-coding RNAs as biomarkers of gallbladder cancer**

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**Figure 2 A and B**

**Figure 3**
Epigenetics and non-coding RNAs in gallbladder cancer

Epigenetics is a widely known mechanism normally implicated in early development (Lyon 1998) and various cellular processes (Reviewed by Berdasco and Esteller 2010). It plays significant role in various human diseases, specifically cancer. Promoter methylation of tumor associated genes is one of the most common epigenetic mechanisms, widely observed in different cancers, including gallbladder cancer (Singh et al., 2012; Singh et al., 2014; Singh et al., 2016; House et al., 2003; Takahashi et al., 2004; Garcia et al., 2009; Roa et al., 2006; Letelier et al., 2014). Very few reports predict the role of methylated miRNAs in gallbladder cancer, but, methylation of IncRNAs is still limited to only one report, that is, promoter methylation of maternally expressed gene 3 (MEG3), a IncRNA, in hepatocellular carcinoma cell (Braconi et al., 2011). Identification of interacting network between miRNA and IncRNA in gallbladder cancer via epigenetic and other mechanisms (Ma et al., 2014; Ma et al., 2015), and also with protein coding genes in epithelial ovarian (Lu et al., 2007) and endometrial cancer (Huang et al., 2009) has raised the possibility to detect the role of IncRNA methylation in gallbladder cancer too. Since non-coding RNAs are also transcribed from the genome from where protein coding RNAs are transcribed, mechanism involved in the latter should also be the same in the former (IncRNAs). It is expected that development of miRNAs and IncRNAs based therapeutics shall become a likely possibility in gallbladder cancer similar to that in other cancers. Promoter methylation of miR-129-2 in endometrial cancer (Huang et al., 2009), miR-9, miR-129, miR-137 in colorectal cancer (Bandres et al., 2009), miR-137 in pharyngeal and laryngeal squamous cell carcinoma (Langevin et al., 2011) and let-7a-3 in epithelial ovarian cancer (Lu et al., 2007) are reported. Interestingly, in hepatocellular carcinoma cells, promoter methylation of IncRNA, MEG3 (Maternally Expressed Gene 3) is associated with down-regulation of miR-29a (Braconi
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et al., 2011). Cells treated with drugs, like 5-Aza-2-deoxycytidine or siRNAs can restore the expression of the MEG3 suggesting an interrelationship between the two non-coding RNAs (miRNA and lncRNA), that is, methylation dependent tissue specific gene regulation of MEG3 by miR-29a. Our recent methylome data from gallbladder cancer and gallstone disease tissues have revealed several differentially methylated miRNAs, which are currently under validation to understand their functional/ regulatory with gallbladder cancer, if any (unpublished data).

Future prospects of developing non-coding RNA based biomarker of gallbladder cancer

The rapidly growing online literatures suggesting the possible roles of miRNA and lncRNA in cancer have raised the expectations on the therapeutic applications of non-coding RNAs in the near future. Continued researches on non-coding RNAs, miRNAs and lncRNAs, in cancer and their interaction adds significantly to the current understanding in cancer biology. (Ma et al., 2015). Non-coding RNAs are now widely known for their regulatory roles in different cellular processes. However, the identification of particular function of ncRNAs seems difficult because of absence or lack of complete functional motifs or domains in ncRNAs. Improved and modern effective techniques for cloning and characterization may be needed to identify appropriate functional cellular targets. Cloning of full length transcripts of these ncRNAs will provide more insights into their functional characteristics following knock-in and knock-out experiments in the animal/mouse or cellular models. Experimental investigations are also needed to understand the spatial-temporal expression of ncRNAs and their specific regulatory functions in cancer, including gallbladder cancer. The therapeutic efficacy of ncRNAs becomes more significant from the reports of clinically relevant and authentic observations on gallbladder cancer cells lines or in vivo mouse models treated with short interfering or lentiviral mediated mimics or inhibitors of ncRNAs or chemotherapeutic drugs or medicinal plant extracts. However, it requires careful
examination or understanding on their role in cell proliferation, differentiation, cycle transition, apoptosis, epithelial to mesenchymal transition, EMT, etc. The main problem is the non-availability of common functional phenotypes (of miRNAs and lncRNAs) in cancers that makes it more difficult to ascertain specific roles of these ncRNAs in gallbladder cancer. The use of molecules or drugs against ncRNAs has increased the number of target molecules further. Use of locked nucleic acids (LNA), short interfering (si-) based small RNAs or anti-lncRNAs have shown their prospects as useful prognostic and therapeutic biomarkers. Emerging evidences of miRNAs and lncRNAs and their disease specific expression patterns are expected to help map or develop signatures of gallbladder cancer (Li et al., 2015; Li and Pu 2015) and also for other cancers (Yu et al., 2015; Zang et al., 2013; Hu et al., 2014). Only then, the early diagnosis will become feasible. Development of mice models of single ncRNAs (miRNAs or lncRNAs) shall be helpful in elucidating altered expression of some of these transcripts in particular type of cancers, such as gallbladder cancer. Upcoming technological developments, like next generation sequencing, whole genome sequencing or RNA seq shall be able to provide a clear picture of the ncRNAs in gallbladder cancer, generating its characteristics, like cis-acting sites, miRNA targets, trans-acting protein coding genes, etc. Thus, the approach towards the current therapeutic scenario of ncRNAs in gallbladder cancer needs improvement to successfully clone the different ncRNA transcripts, characterize (structural and functional) and finally investigate their cellular action in response to novel drugs or other molecules.
References


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(http://dx.doi.org/10.1155/2015/108458).


retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. Mol. Cell 39, 925-938


Running title: **Non-coding RNAs as biomarkers of gallbladder cancer**


Legends

Table 1: Selected list of miRNAs identified for biomarker development in GBC and GSD

Table 2: Selected list of long non-coding RNAs identified for biomarker development in GBC and GSD

Figure 1: Role of miRNA in the gallbladder cancer. Metastatic inhibition (A) and promotion (B, C, D) by miR-182 and miR 20a, pro-apoptotic by miR-146b-5p (E), miR-1, miR-145-5p (J), cell cycle arrest by miR-146b-5p (F) and miR-26a (G), anti-proliferation by miR-26a (I) and pro-proliferation by miR-26a (H) and anti-cell viability and colony formation of gallbladder cancer cells by miR-1 and miR-145-5p (K) are schematically shown. Level of miRNAs (L) and relationship of genetic variants with gallbladder cancer (M) is also given in the lower panel. [Abbreviations: TGFB- Transforming growth factor beta, CADM1- Cell adhesion molecule 1, HMGA2- High mobility group AT hook 2, VEGF A- Vascular endothelium growth factor alpha, AXL- AXL receptor tyrosine kinase]

Figure 2A: Possible mechanistic action of miRNA and IncRNA towards mRNA in gallbladder cancer.

Figure 2B: Gene regulation of HOX gene by lncRNA, HOTAIR

Figure 3: Cellular changes such as metastasis, proliferation and cell cycle transition in gallbladder cancer (A, B, C) by IncRNAs, MALAT1, ITGB1, HOTAIR and KIAA0125. [Abbreviations: MALAT1- Metastasis-associated lung adenocarcinoma transcript 1, ITGB1-Integrin beta-1].
Table 1: Selected list of miRNAs identified for biomarker development in GBC and GSD

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>miRNA Molecules identified</th>
<th>Biopsy type</th>
<th>Technology</th>
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<tbody>
<tr>
<td>1.</td>
<td>miR-26, miR-34a</td>
<td>Tissue/cell line</td>
<td>Validation Assays</td>
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<td>GBC</td>
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<td>2.</td>
<td>miR-146b-5p</td>
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<td>3.</td>
<td>let-7a, miR-21, miR-187, miR-143, miR-202, miR-335</td>
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<td>Microarray</td>
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<td>miR-20a</td>
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<td></td>
<td>Yang et al., 2015</td>
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<tr>
<td></td>
<td>miR-210, miR-200c, miR-194 and miR-192</td>
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<td>11.</td>
<td>miR-26a</td>
<td>Tissue/cell lines</td>
<td>QRT-PCR</td>
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<tr>
<td></td>
<td>GBC</td>
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<tr>
<td></td>
<td>Li et al., 2006</td>
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<tr>
<td>12.</td>
<td>miR-135</td>
<td>Tissue/cell lines</td>
<td>QRT-PCR</td>
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<td>GBC</td>
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<td></td>
<td>Zhou et al., 2015</td>
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<td>13.</td>
<td>miR-196a, miR-499, miR-146a</td>
<td>Tissue</td>
<td>PCR based SNP typing</td>
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<td></td>
<td>GBC</td>
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<td></td>
<td>Srivastava et al., 2008</td>
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<td>14.</td>
<td>miR-27a, miR-570, miR-181a</td>
<td>Tissue</td>
<td>PCR based SNP typing</td>
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<td>GBC</td>
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<td></td>
<td>Gupta et al., 2015</td>
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<td>15.</td>
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</table>
Table 2: Selected list of long non-coding RNAs identified for biomarker development in GBC and GSD

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>IncRNA Molecules identified</th>
<th>Biopsy type identified</th>
<th>Technology</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.</td>
<td>MALAT1 (Metastasis)</td>
<td>Tissue/cell line</td>
<td>QRT-PCR &amp; Validation Assays</td>
<td>GBC cell proliferation, Cell cycle &amp; ERK/MAPK pathway</td>
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<tr>
<td></td>
<td>(associated lung adenocarcinoma transcript 1)</td>
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<td>17.</td>
<td>CCAT1 (Colon cancer)</td>
<td>Tissue/cell line</td>
<td>QRT-PCR &amp; Validation Assays</td>
<td>GBC cell proliferation &amp; migration</td>
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<td></td>
<td>(associated transcript 1)</td>
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<tr>
<td>18.</td>
<td>ITGB1 (Integrin beta 1)</td>
<td>Tissue/cell line</td>
<td>QRT-PCR &amp; Validation Assays</td>
<td>GBC cell proliferation, migration</td>
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<td>19.</td>
<td>HOTAIR</td>
<td>Tissue/cell line</td>
<td>QRT-PCR &amp; Validation Assays</td>
<td>GBC Metastasis, histone methylation, Cell viability, apoptosis</td>
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<td>20.</td>
<td>KIAA0125</td>
<td>Tissue/cell line</td>
<td>QRT-PCR &amp; Validation Assays</td>
<td>GBC cell migration, invasion, epithelial-mesenchymal transitions (EMT)</td>
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</tbody>
</table>
Highlights

1. Role of non-coding RNAs in gallbladder cancer and therapeutic efficacies.
3. Future significance in developing effective target or biomarker(s) for gallbladder cancer.