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 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.

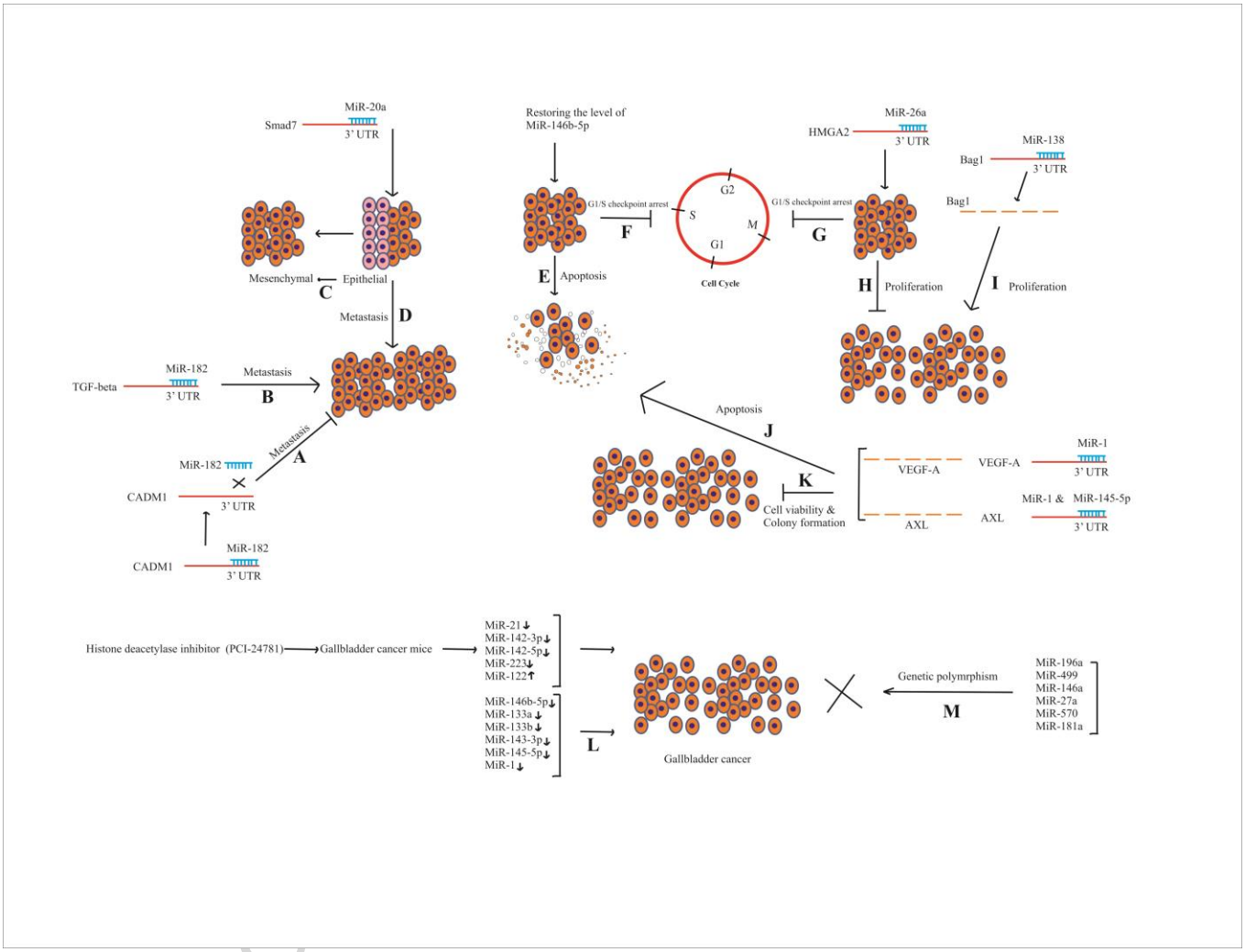

Figure 1

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.

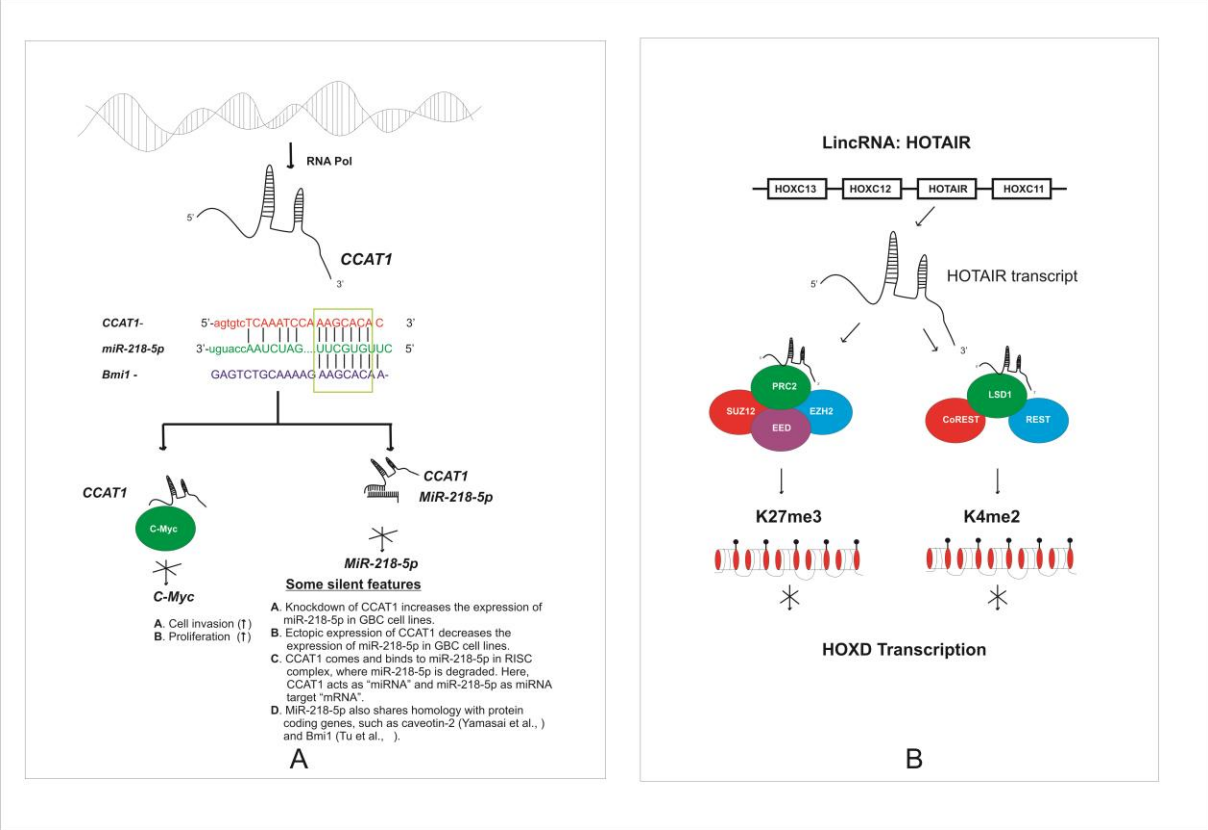

Figure 2 A and B

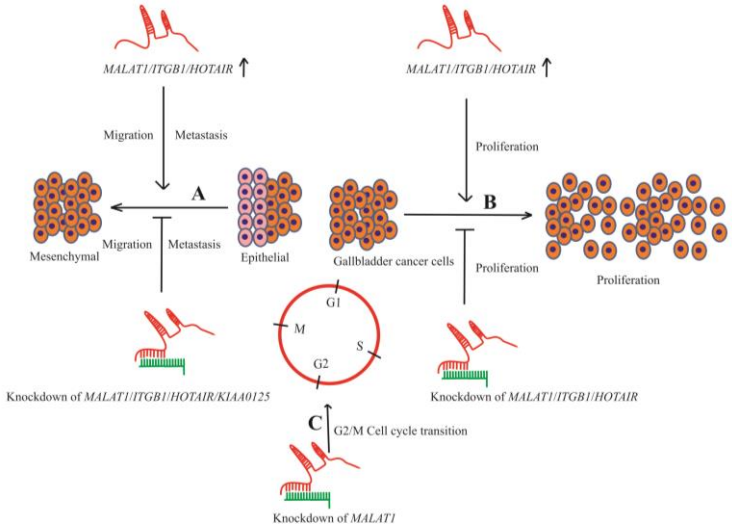

Figure 3

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 lncRNAs is still limited to only one report, that is, promoter methylation of maternally expressed gene 3 (MEG3), a lncRNA, in hepatocellular carcinoma cell (Braconi et al., 2011). Identification of interacting network between miRNA and lncRNA 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 lncRNA 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 (lncRNAs). It is expected that development of miRNAs and lncRNAs 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 lncRNA, *MEG3* (*Maternally Expressed Gene 3*) is associated with down-regulation of *miR-29a* (Braconi

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.

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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 lncRNA towards mRNA in gallbladder cancer.

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

Figure 3: Cellular changes such as metastasis, proliferation and cell cycle transition in gallbladder cancer (A, B, C) by lncRNAs, *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

Sl. No.	miRNA Molecules identified	Biopsy type	Technology	Status	References
1.	*miR-26, miR-34a*	Tissue/cell line	Validation Assays	GBC	Zhou et al., 2014; Jin et al., 2014
2.					
3.	*miR-146b-5p*	Tissue/cell lines	Validation assay	GBC	Cai et al., 2015
4.	*let-7a, miR-21, miR-187,*	Blood Serum	Microarray	GBC	Li and Pu 2015
	miR-143, miR-202, and miR-335				
5.	*miR-20a*	Tissue	miRNA Library screening	GBC	Chang et al., 2013
			by High Content Screening		
6.	*miR-1, miR-133*	Tissue	Microarray & RTPCR	GBC	Letelier et al., 2014
	miR-143, miR-145				
7.	*miR-21, miR-142-3p, miR-122*	Mice Tissue	Microarray & RTPCR	GBC	Kitamura et al. 2012
	miR-142-5p, miR-223				
8.	*miR-138*	Tissue/cell lines	QRT-PCR	GBC	Ma et al., 2015
9.	*miR-155*	Tissue/cell lines	QRT-PCR	GBC	Kono et al., 2013
10.	*miR-182*	Tissue/cell lines	QRT-PCR	GBC	Qiu et al., 2014
11.	*miR- 133a and miR-891a*	Tissue	Whole genome sequencing	GSD	Yang et al., 2015
	miR-210, miR-200c, miR-194 and *miR-192*		& QRT-PCR		
12.	*miR-26a*	Tissue/cell lines	QRT-PCR	GBC	Li et al., 2006
13.	*miR-135*	Tissue/cell lines	QRT-PCR	GBC	Zhou et al., 2015
14.	*miR-196a, miR-499, miR-146a*	Tissue	PCR based SNP typing	GBC	Srivastava et al., 2008
15.	*miR-27a, miR-570, miR-181a*	Tissue	PCR based SNP typing	GBC	Gupta et al., 2015

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

Sl. No.	lncRNA Molecules identified	Identified function	Biopsy type	References	Technology	Status	
16.	*MALAT1* (*Metastasis -associated lung adeno carcinoma transcript 1*)	proliferation, Cell cycle & ERK/MAPK pathway	Tissue/cell line	Wu et al., 2014	QRT-PCR &Validation Assays	GBC	Cell
17.	*CCAT1 (Colon cancer associated transcript 1)*	proliferation & migration	Tissue/cell line	Ma et al., 2015	QRT-PCR &Validation Assays	GBC	Cell
18.	*ITGB1 (Integrin beta 1)*	proliferation, migration Invasion	Tissue/cell line	Wang et al. 2015	QRT-PCR &Validation Assays	GBC	Cell
19.	*HOTAIR*	Metastasis, histone methylation, viability, apoptosis	Tissue/cell line	Rajnis et al.,2010 Yang et al., 2011	QRT-PCR &Validation Assays	GBC	Cell
20.	*KIAA0125*	migration, invasion, epithelial-mesenchymal transitions (EMT)	Tissue/cell line	Lv et al., 2015	QRT-PCR &Validation Assays	GBC	cell and

Highlights

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