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Hepatic MicroRNA Orchestra: A New Diagnostic, Prognostic and Theranostic Tool for Hepatocarcinogenesis

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Abstract: MicroRNAs are evolutionary conserved single stranded non-coding RNAs with immense ability to post-transcriptionally regulate gene expression via complementary base pairing with mRNAs of more than 50% protein encoding genes. They play diverse roles in physiological and pathophysiological processes such as normal development and cancer pathogenesis respectively. Recent investigations have focused on the identification and characterization of microRNAs aberrantly expressed in cancer and their target molecules that are critically involved in the initiation, progression and development of carcinogenesis as possible diagnostic, prognostic and theranostic (Integration of biomarker use as diagnostic tools and target-specific therapies to enhance selective and individualized therapy) tools to augment conventional cancer therapeutic armamentarium. In this mini review, we bring to focus the intricate interactions between microRNAs aberrantly expressed in hepatocarcinogenesis and their interactions with the transforming growth factor beta (TGF-β)/Small mother against decapentaplegic (Smad) and the mitogen activated protein kinases (MAPKs) signaling pathways. Importantly, we highlight microRNA-Specific targets as possible biomarkers for prognosis, diagnosis and as therapeutic targets for hepatocellular carcinoma (HCC) while at the same time exploring new directions for future investigations.

Keywords: Hepatocarcinogenesis, MAPK, MicroRNA Therapeutics, TGF-β, Smad.

INTRODUCTION

MicroRNAs are small evolutionary conserved single stranded endogenous non-coding RNAs [1-4] first discovered in 1993 [5, 6] and later named as microRNAs [7-9]. Essentially, advancement in molecular cell biology and cancer biology have uncovered important regulatory functions of microRNA in diverse cellular processes spanning developmental timing, cell differentiation and metabolism [3, 10] through post-transcriptional regulation of translation and protein synthesis. With the help of high throughput techniques including microarrays or next-generation sequencing, it has been shown that microRNAs are variably dysregulated in almost all known human cancers [11, 12] and they offer novel opportunities for diagnostic, prognostic and theranostic (Integration of biomarker use as diagnostic tools and target-specific therapies to enhance selective and individualized therapy) application in human cancer treatment [13-16]. Already, some microRNA expression patterns are being used as diagnostic screening tools for accurate identification of different cancer types and their tissue origins [11]. What even makes the microRNA revolution indispensable in current cancer research and therapeutics is the fact that 30% of human protein encoding genes are estimated to be post-transcriptionally regulated by microRNAs [10]. In fact, 30-50% of microRNAs are reportedly encoded within the introns of protein coding genes with the remaining microRNAs said to be located in intergenic sites [17]. Clearly, expanding our knowledge and understanding of microRNA diversity in the context of their regulatory roles in normal homeostatic processes, cancer and other disease pathologies will certainly open up a new therapeutic goldmine for the exploration of novel therapies to augment existing therapeutic armamentarium.

To this end, there have been a number of expert reviews on microRNAs and their subtle and intricate molecular interactions with many signaling pathways implicated in human cancers [18-26]. For instance, Davis *et al.* [1] and Hata and Davis [10] have reviewed the regulation of microRNA biogenesis by Smad proteins (Signal transducers and transcriptional effectors of the TGF-β signaling) and the TGF-β pathway in many human cancer subtypes. As an extension with specific emphasis on hepatocarcinogenesis, we herein highlight the intricate interactions and cross signaling between the TGF-β/Smad signaling, the MAPK pathway and microRNAs aberrantly expressed in hepatocarcinogenesis. Specifically, we discuss the biogenesis of microRNAs, their functional roles, targets, crosstalk with the TGF-β/Smad and the MAPK pathways in HCC, microRNA therapeutics, challenges of microRNA research and future expectations.

MICRORNA BIOGENESIS

MicroRNA biogenesis in an oversimplified view, may involve six closely regulated stages: transcription of the microRNA gene, nuclear processing of primary microRNA into precursor microRNA by Drosha-DGCR8 complex,

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nucleo-cytoplasmic export of precursor microRNA, cytoplasmic cleavage of precursor microRNA by Dicer (An RNase enzyme) into a double stranded microRNA, strand selection, and specific mRNA targeting. Two important enzymes required for microRNA biogenesis are DROSHA and DICER, found in the nucleus and cytoplasm respectively. After transcription of microRNA encoding genes by RNA pol II, the primary microRNA transcript (Pri-microRNA) formed is processed by DROSHA-DGCR8 into a 65-80 nucleotides (nt) hairpin structure known as precursor-miRNA (Pre-miRNA) in the nucleus. DROSHA-DGCR8 complex is required for the stability and integrity of the DROSHA-DGCR8-pri-microRNA cleavage. Indeed, the precise position and orientation of DROSHA-DGCR8 cleavage in the generation of pre-microRNA is crucial for successful biosynthesis and structural and functional quality of a particular microRNA. This is because an error in the DROSHA cleavage site could possibly lead to a number of errors with far reaching consequences including misidentification of both 5' and 3' nt identities of the mature microRNA, changes in microRNA seed sequence, redirection of microRNA target, and inversion of the relative stability of the two microRNA strands (Mature microRNA guide strand and microRNA passenger strand) and there is also a possibility of incorporating an improper microRNA unto the RNA induced silencing complex (RISC) complex [10]. By the activities of Exportin-5 and RAN-GTP the pre-microRNA is exported into the cytoplasm where DICER (Second RNase III enzyme) processes the pre-microRNA into a double-stranded 22 nt product comprising a mature microRNA guide strand and microRNA passenger strand. While the microRNA guide strand is loaded onto the RISC, on the other hand, the passenger strand degrades. The RISC-mature microRNA complex is then guided to their target mRNAs through interaction with various Argonaut families (Ago1-Ago4). MicroRNA-dependent silencing or activation of target mRNAs may involve many mechanisms. It is posited that partial complementary paring of microRNA/RISC complex with the 3' untranslated region (UTR) of a target mRNA could lead to repression of translation [27], degradation of the target mRNA [28] ultimately leading to target gene silencing [4, 29] or activation of target mRNA [18]. A number of microRNAs can be processed *in vitro* by DROSHA-DGCR8 complex, but processing of pri-microRNA to pre-microRNA *in vitro* is slow in kinetics, an observation suggestive of probable involvement of accessory factors. In pursuit of these missing links (Accessory factors), the dead-box RNA helicases p68 (DDX5) and p72 (DDX7) were identified as a part of the DROSHA processing complex [10]. Interestingly, p68 and p72 were reported to associate with DGCR8 [30, 31]. To confirm the involvement of p68 and p72 in microRNA biogenesis, it was shown that steady state levels of mature microRNA in p68-null and p72-null mouse embryonic fibroblasts (MEFs) decreased significantly compared with controls indicative of the crucial role of p68 and p72 in microRNA biosynthesis [10]. Each of the stages of microRNA biogenesis could serve as a potential target for crosstalk with many signaling molecules and pathways dysregulated in cancer. So we ask the question: How can epigenetic manipulation of precursors (Argonaut family of proteins, DGCR8, p68, p72) and enzymes (DROSHA and DICER) required for microRNA biogenesis be used to silence oncomirs (MicroRNAs associated with cancer) while at the same time enhance the expression of tumor suppressor (Factors that oppose carcinogenesis and are generally downregulated in tumors compared to non-tumorous areas) microRNAs in hepatocarcinogenesis?

TARGET GENES OF MICRORNAS

Molecular pathways so far identified to play crucial roles in hepatocarcinogenesis may include multiple tyrosine kinase growth factor ligands including TGF-β, EGF, HGF, FGF, VEGF and cell cycle regulators such as p53, c-Myc, Cyclin D1, Wnt/β-catenin signaling pathway [32-35]. Interestingly, microRNAs aberrantly expressed in hepatocarcinogenesis either promote or suppress phenotypic hallmarks of cancer by targeting the same genes that drive the molecular pathways implicated in hepatocarcinogenesis. Indeed, microRNA targets generally may be grouped into two as: tumor suppressor genes and oncogenes and can also be designated as predicted or validated based on whether it has been predicted by a computational method or experimentally confirmed respectively. For example, four computational methods (Miranda, TargetScan, PicTar and RNA22) have all predicted programmed cell death protein 4 (PDCD4), a neoplastic transformation inhibitor of both humans and mouse to be a target of miR-21 [36]. In this section we briefly discuss microRNA targets in the light of oncogenes and tumor suppressor genes that functionally affect microRNA expression patterns in hepatocarcinogenesis through upregulation or downregulation.

In normal physiological state, there exists a balance between cell proliferation and apoptosis (Programmed cell death) and this balance is closely monitored by regulatory pathways. However in hepatocarcinogenesis, this homeostatic balance is disrupted through derangement of the regulatory pathways resulting in cells growing out of control and all the associated phenotypic consequences. Apoptosis is one of the major cellular mechanisms that regulate cell growth and proliferation together with other cell cycle regulators. A number of tumor suppressor genes and oncogenes interplay to regulate apoptosis but they are in turn targeted by microRNAs implicated in hepatocarcinogenesis. Intrinsic apoptotic pathways are exclusively mitochondria-mediated and are driven by pro-apoptotic (Bmf and Bim) and anti-apoptotic (Bcl-2, Mcl-1 and Bcl-w) Bcl-2 family of proteins [26]. Indeed, the net apoptotic output of mitochondria-mediated apoptosis is a function of the combined actions of pro-apoptotic and anti-apoptotic Bcl-2 proteins. While miR-221 and miR-25 target Bmf and Bim to reduce Bmf/Bim-mediated apoptosis to favor tumor cell survival; miR-29, miR-101 and miR-122 on the other hand target Bcl-2, Mcl-1 and Bcl-w respectively to increase apoptosis. Yoon *et al*. and Law and Wong [26, 37] have reported from an *in vitro* study that miR-491-5p could increase sensitivity of HCC cells to TNF-α-induced apoptosis. In that same study, Yoon and colleagues have indicated that miR-491-5p might have possibly targeted its predicted targets (α-fetoprotein, heat shock protein-90 and nuclear factor-kappa B [NF-κB) to increase death receptor-mediated apoptosis. Also, p53, a key pro-apoptotic factor is almost totally mutated in half of all known human cancers including HCC [20] and it has been a

major target of many microRNAs aberrantly expressed in HCC. For instance, in a clinical study, 76% of HCC patients diagnosed with mutated p53, showed decreased expression of miR-34a [38] indicating that miR-34a could probably be a tumor suppressor that enhances the expression of p53 to disrupt anti-apoptotic signals from tumor cells. Similarly, Bim, another pro-apoptotic factor has long been suspected to be dysregulated in HCC. Frankel *et al.* [39] have reported hepatocyte apoptosis in liver-specific Dicer deleted-mice and they attributed their observation to silence Bim [40]. p21/Cipl, a cyclin-dependent kinase inhibitor was targeted by miR-17-92 cluster which have consistently been shown upregulated in HCC [41].

Also, other cell cycle regulatory factors (Cyclin D1, Cyclin D2, CDK6, E2, E2F3, CDKN1B/p27/Kipl, and CDKN1C/p57/Kipl) were reportedly targeted by microRNAs thereby playing crucial roles in cell cycle regulation, especially at the G_1/S transit point. For example, many reports have shown cell cycle gene regulation by microRNAs including miR-221 targeting CDKN1B/p27/Kipl and CDKN1C/p57/Kipl (CDK inhibitors) in HCC cells [42], miR-106b and miR-93 targeting E2F1 [43], miR-25 targeting Bim [43], miR-223 targeting stathmin1 [44], and miR-26a targeting G_1/S cyclins (Cyclin D2 and E2) in murine liver cancer [45].

Recently, several reports have shown PDCD4 as a functional target of miR-21 in a number of cancer phenotypic markers including cell proliferation, invasion, metastasis and neoplastic transformation [36, 46, 47]. miR-183 was also shown to inhibit TGF-β_1-induced apoptosis in human HCC by silencing PDCD4 [48]. Further, PDCD4 was shown to negatively modulate AP-1 [49] thereby interfering with the activation of AP-1-led transcription and tumor progression in transgenic mice [50].

Mammalian Sprouty is a family of four (Spry 1-4) proteins that function as tumor suppressors; they are normally downregulated in cancers including HCC [51, 52]. Importantly, Sprouty2 was shown to negatively modulate the Ras/ERK pathway to abrogate tumor progression, but oncogenic miR-21 however directly targets Sprouty2 in HCC to promote hepatocarcinogenesis [51, 53, 54].

Phosphatidylinositol 3-kinase (PI3K) activates Akt to sustain tumor cell survival, however phosphatase and tensin homolog (PTEN), a tumor suppressor gene which inhibits activation of PI3K/Akt and by acting as an inhibitor of Akt was reported as a target of many microRNAs in a number of cancer subtypes including HCC [55]. For example, PTEN was shown to be a target of miR-21 in HCC [23]. Reportedly, miR-17-92 was said to be overexpressed in many cancers including HCC [20]. Relatedly, estrogen-dependent signaling has been implicated in some cancer subtypes, however in HCC it acts as a tumor suppressor gene [20]. This perhaps explains the reason behind low incidence of HCC in women compared to men. Evidently, miR-18a was shown to target estrogen receptor-α, to compromise the tumor suppressor effects of estrogen in HCC among women [56].

Epithelial-mesenchymal-transition (EMT) is a biological process by which a polarized cell anchored to a basement membrane switches to a mesenchymal phenotype acquiring invasive and motility properties [57]. During this process, basement membrane degradation occurs; cells lose E-cadherin, zona occluden-1, β4 integrin and produce fibroblast markers such as vimentin, N-cadherin and α -smooth muscle actin which are already being used as prognostic markers for liver fibrosis, cirrhosis and HCC risk assessment. Though EMT has important functions in embryogenesis, wound healing, tissue remodeling and repair, and tissue morphogenesis, however it has been implicated in the acquisition of metastatic properties (Enhanced invasive and migratory capacity) by tumor cells [58] in a number of cancers [59]. Accordingly, EMT is crucial for the initiation and progression of hepatocarcinogenesis and this has been demonstrated in several *in vitro* and *in vivo* studies [60]. For instance, a number of microRNAs (miR-32, miR-137, miR-346, miR-136, miR-192, miR-210, miR-211) including miR-21 have been predicted to play a key role in TGF-β_1-induced EMT [55]. Inhibition of miR-155 was sufficient to abrogate TGF-β_1-induced EMT in HCC [61]. MiR-216a/217 was shown to have induced EMT and drug resistance in liver cancer by targeting PTEN and inhibitory Smad7 [62]. Also, miR-26b was shown to inhibit EMT in HCC cells by targeting USP9X [63]. In like manner, Smad7, a TGF-β_1-specific receptor mediated Smad2/3 phosphorylation inhibitor, was reported as a direct target of miR-21 in liver cancer [55, 64].

MicroRNAs as stated earlier may also target oncogenes either to promote their pro-tumorigenic functions or counter their expressions and oncogenic functions. For instance, the expression of the pioneer cancer gene, Ras as well as c-Myc show inverse relationship with let-7 expression in HCC. Let-7 was shown to be down-regulated in HCC compared to cirrhosis [24] and it is suspected that this pattern of expression will correlate with an increased expression of Ras and c-Myc. Similarly, miR-34 was reported to have shown an inverse relationship with a number of oncogenes in HCC. For example the expression of miR-34 was restored as that of the oncogenes decreased and vice versa.

Interestingly, Bcl-2, N-Myc, Met, cyclin E_2 (CCNE2) and cyclin-dependent kinase 4 (CDK4) have all been shown to display inverse relationship with miR-34 expression [20, 65-67]. Stathmin, a microtubule protein regulator was reported to promote cell proliferation [68]. This stathmin inversely relates to miR-223 in HCC. miR-223 expression was shown to have decreased in HCC while stathmin was overexpressed [68, 69]. By similar mechanism, miR-1 was reported to have targeted c-Met, histone deacetylases 4 (HDAC4) in HCC, though it was itself silenced by cytosine-guanine dinucleotide via methylation [70].

Another oncogenic factor, STAT3 (Signal transducer and activator of transcription 3) a member of a family of proteins that maintain immune tolerance in tumor cells leading to tumor survival [71] was shown to be a target of some microRNAs in HCC including miR-93. MiR-93 negatively regulates STAT3 [72] thereby increasing the sensitivity of tumor cells to immune surveillance in HCC.

There is a probable inverse relationship between microRNAs aberrantly expressed and their targets, and it is tempting to say that tumor cells may compromise the cellular

homeostatic machinery to their advantage by using oncomirs as a decoy. Once tumor cells succeed in usurping the power of cellular regulation, carcinogenesis proceeds with total disregard to surrounding cells and tissues. Though tumor suppressor genes including microRNAs repressed in cancer mount defense to restore homeostatic balance but evidently it is not sufficient as the oncogenes win the fight.

REGULATORY FUNCTIONS OF MICRORNAs

Conventionally, microRNA-mRNA interaction leads to translational repression or mRNA cleavage which eventually results in decrease in protein output of the target gene [73], and this has long been the traditional understanding of microRNA regulatory function. However, recent insights into the biology of microRNAs have not only disputed the traditional understanding of microRNAs as negative regulators of gene expression but have shown evidence that they can also increase the protein output of their targets. In this light, Vasudevan *et al.* [74] have shown evidence of increase in the translation of target mRNA by specific microRNAs through recruitment of protein complexes to AU-rich elements of the target mRNA. Similarly, Eiring *et al.* [75] have reported that microRNAs may indirectly increase the protein output of their target mRNA by de-repressing proteins that block translation of the target gene. Further, microRNAs can cause global protein synthesis by facilitating ribosome biogenesis [76] or better still switch their regulatory function from translational repression to translational activation of their target gene through cell cycle arrest [74]. MicroRNA-dependent mechanisms that increase protein output of their target mRNAs could be employed to enhance expression of microRNAs that function as tumor suppressors.

Also, microRNAs out of their cells of origin abundantly circulate in blood, indicating that they can be transported to distant sites to affect target cells perhaps in a hormone-like fashion. Like hormones, microRNAs have been reported to regulate both short-and long-term cell-cell communication [77]. Indeed, microRNAs have diverse regulatory functions because of the diversity of their conserved targets. Many models have been proposed to rationalize the plethora of regulatory functions of microRNAs. One of such models named: abundance, differential expression, and targeting promiscuity of metazoan microRNAs [4] tries to put microRNA functional diversity into proper perspective. Accordingly, many mechanisms have been proposed including switch interactions, tuning interactions, neutral interactions to help explain the functional diversities inherent in microRNAs as reviewed in [78] and elsewhere [73].

It has been posited that microRNAs function as biological rheostats to modulate expression of target genes rather than in a strict on/or off manner [4]. This possibly implies that the function of microRNAs may not only be cell type and context-dependent but also flexible enough to allow for changes in cellular microenvironment. It is not surprising that one type of microRNA may show varied expressions patterns in different cell types. The functional impact of microRNAs may not be limited to their target mRNA but may also affect downstream targets of their target genes thereby influencing a whole signaling cascade. For instance during hepatocarcinogenesis miR-24 and miR-629 were reported to be overexpressed following inactivation of hepatocyte necrosis factor 4α (HNF-4α) through interleukin-6-dependent signaling via STAT3 [79] but any mechanism that depletes interleukin-6 or STAT3 may up-regulate HNF-4α to reverse the overexpression of miR-24 and miR-629. Sustained expression of these two microRNAs promotes oncogenesis [80] whiles at the same time keeping HNF-4α inactivated. Depending on the functional significance of a particular microRNA in the executive cellular decision-making, its overexpression or down-regulation may affect important cellular processes. A body of evidence shows that a single microRNA may target several hundreds of coding genes [81]. In this case, whatever happens to such microRNA in terms of its expression and function exerts far-reaching consequences on its target mRNAs and the roles they play. MicroRNAs form an integral component of all the regulatory functions of the body, therefore the more we increase our understanding of microRNA biology in the context of cancer pathogenesis the closer we come to the very underpinnings of cancer.

ROLE OF LIVER-SPECIFIC MICRORNAs

Some liver-specific microRNAs play important roles in both normal physiology and pathophysiology of the liver [82]. For example, miR-122 was shown to be liver-specific and plays important roles in normal liver development; however its dysregulation or down-regulation in murine models of liver disease have been linked to decrease in the levels of cholesterol and lipid-metabolizing enzymes [83] possibly increasing the risk of fatty liver disease. Similarly, miR-181 modulates GATA6, a transcriptional factor that control liver organogenesis [84]. Also, a number of microRNAs including miR-130, miR-29 and let-7 have all been shown to play various roles in normal liver development [18]. HCC is the most debilitating liver disease which begins as chronic liver inflammation resulting in liver fibrosis then progresses to cirrhosis increasing the risk of HCC.

With specific regard to the liver, microRNAs have been shown to display expression patterns reflective of the progression of hepatocarcinogenesis. For instance, from liver fibrosis through cirrhosis to HCC, a number of microRNAs have been shown to display varied expression patterns (Table **1**). Generally but not universally, the expression of microRNAs that function as tumor suppressor genes is frequently reduced in tumor samples relative to normal tissues, suggesting the potential role of the microRNAs in maintaining the differentiated state [10]. For example, the following microRNA panels (miR-223, miR-214, miR-200b, miR-199b, miR-199a*, miR-199a, miR-150, miR-139, miR-101 and miR-18) were reported to be highly expressed in benign tissues in human HCC than tumorous areas, however, miR-21, miR-33, miR-130b, and miR-221 were shown to be overexpressed in tumor areas than the adjacent benign areas [32]. These differential expression patterns of hepatic microRNAs seem to reinforce their importance as biomarkers for HCC risk assessment, diagnosis, staging and identification.

Reportedly, a number of microRNA subsets including miR-21, miR-199a and miR-155 were shown to be highly expressed in a number of tumors and may promote tumor growth through inhibition of pro-apoptotic pathways [10].

Table 1. Some microRNA expression patterns in hepatocarcinogenesis.

microRNA	Liver Disease	Pattern of Expression	Proposed mechanism	References
miR-133a	Liver fibrosis	Down-regulated	It mediates TGF-β_1-dependent de-repression of collagen synthesis in HSC during liver fibrosis	[85]
miR-29	Liver fibrosis	Down-regulated	TGF-β_1 and NF- kB-dependent down-regulation of miR-29 in HSC leading to ECM increase	[86]
miR-221[a]/222[a]	Liver fibrosis	Upregulated	Activation of HSC to promote fibrogenesis	[87]
miR-196	Liver fibrosis	Down-regulated	Inhibits TGF-β_1-dependent activation of HSC	[88]
miR-214-5p	Liver fibrosis	Upregulated	Activate HSC to promote fibrogenesis	[89]
miR-146a	Liver fibrosis	Down-regulated	Negatively modulates TGF-β_1-dependent activation of HSC by inhibiting HSC proliferation but promoting HSC apoptosis	[90]
miR-21[a]	Liver fibrosis	Upregulated	Enhances TGF-β_1-dependent fibrogenic potential through Smad7 down-regulation	[64]
miR-194	Liver cancer	Down-regulated	Inhibits metastasis of cancer cells	[91]
miR-150	Liver fibrosis	Down-regulated	Suppresses activation of HSC	[92]
miR-335	Liver fibrosis	Down-regulated	Inhibits HSC migration and activation	[93]
miR-155	Alcoholic liver disease	Upregulated	Promotes production of TNF-α by Kupffer cells	[94]
miR-199	Liver fibrosis	Upregulated	Promotes progression of liver fibrosis by activating HSC	[18]
miR-200	Liver fibrosis	Upregulated	Activates HSC cells	[18]
miR-34a	Non-alcoholic fatty liver disease	Upregulated	-	[95]
miR-885-5p	Cirrhosis	Upregulated	-	[96]
miR-122[a]	Liver steatosis	Upregulated	-	[95]
miR-101[b], miR-145[b], miR-199b	HBV infection	Down-regulated	-	[97]
miR-143, miR-122[a], miR-22[c], miR-99a, miR-21[a], miR-25, miR-375	HBV infection	Upregulated	-	[97]
miR-29a	HBV, HCC	Upregulated	HBX promotes cell migration in HCC via targeting PTEN	[98]
miR-17/92 cluster	HCC	Upregulated	Promotes HeG2 cell proliferation	[99]
miR-181b	HCC	Upregulated	Promotes hepato-carcinogenesis through TIMP3	[100]
miR-23a/27a/24 cluster	HCC	Upregulated	-	[101]
miR-375	HCC	Downregulated	Suppress HCC cell growth via AEG-1 silencing, G1/S arrest	[102]
miR-145[b]	HCC	Downregulated	Acts as a tumor suppressor via silencing of HDAC2	[103]
miR-22[c]	HCC	Downregulated	Anti-proliferative on HCC cells	[104]
miR-18a	HCC	Upregulated	Suppresses ERα to promote HCC in women	[56]
miR-520e	HCC	Down-regulated	Suppresses HCC cell growth via NF-kB-inducing kinase modulation	[105]
miR-101[b]	HCC	Down-regulated	Pro-apoptotic effect on HCC cells	[106]

(Table 1) Contd….

microRNA	Liver Disease	Pattern of Expression	Proposed mechanism	References
miR-29	HCC	Down-regulated	Sensitizes HCC cells to apoptotic machinery via silencing of Bcl-2 and Mcl-1	[107]
miR-195	HCC	Down-regulated	Suppresses tumorigenesis by regulating G1/S transition of HCC cells	[108]
miR-16	HCC	Down-regulated	Silences COX-2 expression in HCC	[109]
miR-21[a]	HCC	Upregulated	Suppresses PTEN and hSulf-1 expression and promote HCC progression via Akt/ERK pathway	[110]
miR-124	HCC	Down-regulated	Suppresses HCC cell proliferation via targeting STAT3	[111]
miR-145[b]	HCC	Down-regulated	Inhibits HepG2 cell motility, proliferation via IRS1	[112]
miR-145[b]	HCC	Down-regulated	Modulates IGF; arrest G (2) –M cell cycle, targets IRS1 and IRS2	[113]
miR-214	HCC	Down-regulated	Targets β-catenin pathway to suppress invasion of HCC cells; inhibits c-Myc, cyclin D1, TCF-1, LEF-1	[114]
miR-222[a]	HCC	Upregulated	Confers migratory potential on HCC cells via activation of Akt pathway	[115]
miR-449	HCC	Down-regulated	Deregulates HDAC2 in hepatocytes	[116]
miR-122a	HCC	Down-regulated	Modulates cyclin G1	[24]
miR-21[a], miR-31, miR-122, miR-221[a], miR-222[a]	HCC tissues	Upregulated	-	[117-119]
miR-145[b], miR-146a, miR-200c, miR-223	HCC tissues	Down-regulated	-	[23, 117]
miR-21[a]	HCC	Upregulated	Promotes HepG2 cell proliferation via repression of MAP2K3 and PTEN	[120, 121]
miR-23a	Liver cancer	Upregulated	Predicted to target SEMA6D, POU4F2, NEK6, SLC6A14	[121]
miR-223	HCC, Chronic hepatitis	Upregulated	-	[119]
miR-199a/b-3p	HCC	Down-regulated	Inhibits HCC cell growth via silencing of PAK4-Raf-MEK-ERK pathway	[122]

[a]Consistently overexpressed in hepatocarcinogenesis; [b] Consistently downregulated in hepatocarcinogenesis; [c] Inconsistently expressed in hepatocarcinogenesis. Abbreviations: AEG-1 (Astrocyte elevated gene-1), COX-2 (Cyclo-oxygenase-2), ECM (Extracellular matrix), ERK (Extracellular signal-regulated kinase), HCC (Hepatocellular carcinoma), HSC (Hepatic stellate cells), HDAC2 (Histone deacetylases 2), hSulf-1 (Human sulfatase 1), IGF (Insulin growth factor), IRS1 (Insulin receptor substrate 1), IRS2 (Insulin receptor substrate 2), LEF-1 (Lymphoid enhancer-binding factor 1), NEK6 (Homo sapien NIMA[never in mitosis gene a]-related kinase 6), NF-Kb (Nuclear factor kappa B), PAK4 (p21-activated kinase 4), TCF-1 (Transcription factor 7), TGF-β (Transforming growth factor β), TIMP3 (Tissue inhibitor of metalloproteinase-3), TNF-α (Tumor necrosis factor alpha), POU4F2 (Homo sapien POU domain, class 4, transcription factor 2), PTEN (Phosphatase tensin homologue), SEMA6D (Homo sapien sema domain, transmembrane domain [TM] and cytoplasmic domain, [semaphoring] 6D), SLC6A14 (Homo sapien solute carrier family 6, [neurotransmitter transporter], membrane 14), STAT3 (Signal transducer and activator of transcription 3).

Elsewhere microRNAs overexpressed or repressed in hepatocarcinogenesis have been reviewed [25]. Consistently, miR-21 was shown to be overexpressed in liver fibrosis, cirrhosis and HCC [18], a trend which perhaps emphasizes that miR-21 could be considered as one of many oncomirs in hepatocarcinogenesis and could be used as a biomarker for general screening purposes .But the difficulty with microR-21 is that it has been shown to be overexpressed in a number of other human cancers therefore may not be sufficiently specific enough for early screening of hepatocarcinogenesis. Similarly, microRNAs repressed in hepatocarcinogenesis could also be considered for use as screening tools provided they are liver-specific. It may be suggested that a carefully characterized liver-specific microRNA signatures of which many have been suggested recently, may be needed for quick and accurate early screening and diagnosis of hepatocarcinogenesis. This may probably help to reduce the number of late presentation of HCC cases particularly in

endemic areas. This may be necessary because delayed presentation of HCC cases at late stage limits the range of treatment options to surgical removal which speculatively has low success and survival rates.

INTRICATE INTERACTIONS BETWEEN MICRORNAs AND THE TGF-B_1 SIGNALING IN HEPATOCARCINOGENESIS

TGF-β_1 is a ubiquitous cytokine central in all homeostatic, regulatory and embryonic cellular functions in eukaryotic organisms. Precisely, among the TGF-β family, TGF-β_1 is the most studied and the most implicated in both physiological and pathophysiological processes such as hepatocarcinogenesis. TGF-β_1 employs two serine/threonine receptor kinases known as TGF-β_1 type 1 and 2 receptors (TβR1 and TβR2) for all its receptor-related signaling transduction. Upon ligand stimulation of the constitutive TβR2, it then phosphorylates the TβR1 which in turn phosphorylates the receptor mediated Smads (Smad2 and Smad3 specific for TGF-β_1 and Smad1 and Smad5 specific for BMP). The phosphorylated Smads (pSmad2/3) form complexes with a common Smad (Smad4) then translocate into the nucleus where they partake in the transcription of sequence specific target genes, mostly oncogenes in late phase of hepatocarcinogenesis. TGF-β_1 has been shown in several reports to induce the expression of a number of microRNAs in HCC [100, 123, 124]. For instance miR-23a-27a-24 cluster transfected into Huh7 cells succeeded in abrogating TGF-β_1-induced anti-proliferative and pro-apoptotic effects [26]. Prior to this, TGF-β/Smad signaling plays an important role as a tumor suppressor in early phases of hepatocarcinogenesis, for example PDCD4, a pro-apoptotic molecule was shown to be crucial for TGF-β_1-induced apoptosis of human HCC cells [125], a process important for reducing tumor cell number and survival to maintain cellular homeostasis. Unfortunately, tumor cells can compromise the tumor suppressor effects of TGF-β/Smad signaling, by switching TGF-β signaling from tumor suppression to tumor promotion. For instance tumor cells have been shown to disrupt TGF-β_1/PDCD4-dependent apoptosis of Huh7 cells via miR-183-induced down-regulation of PDCD4 [48]. This functional switch in TGF-β/Smad signaling in HCC primarily involves tumor cells rendering TGF-β_1 totally unresponsive to tumor suppressor signals but having increased sensitivity to oncogenic signals. Meanwhile, the processing and expression of microRNAs in normal physiological and disease states such as hepatocarcinogenesis are strictly regulated at multi-levels by various regulatory pathways including TGF-β/Smad signaling and MAPK (ERK, JNK and p38) pathway. Recent insights into the study of TGF-β signaling shows that it transmits signals to both normal and tumor cells mainly through the central Smad liaison pathways and partly via non-Smad signaling pathways including the MAPK and PI3K pathways. These multi-interactions regulate the overall quantitative output of TGF-β/Smad signaling, and also offer platforms for crosstalk with other signal transduction pathways to determine cell fate. Accordingly, we discuss some of the interactions between dysregulated TGF-β/Smad signaling and aberrant microRNA expression patterns in hepatocarcinogenesis.

Some microRNAs interact with dysregulated TGF-β signaling pathway to either abrogate hepatocarcinogenesis or promote it. Indeed, these interactions can occur at various stages of TGF-β signaling. Some of these interactions occur at TGF-β-specific receptor (TβR1 and TβR2) level. For example, TGF-β-induced EMT in HCC was shown to be suppressed by miR-655 through blockade of TβR2 and ZEB1 [126]. MiR-590-5p cluster functioning as a tumor suppressor gene was shown to regulate cell proliferation and invasion in HCC via TβR2-specific receptor blockade [127]. Also, miR-140-5p cluster was reported to have suppressed tumor growth and metastasis in HCC cells through blockade of TβR1 [128]. Indeed, microRNA/TGF-β interaction is not only restricted to TGF-β-specific receptors. Other microRNAs also directly modulate TGF-β signaling pathway independently of its receptors. Notably, miR-302b and miR-20a, were shown in rodent models of HCC to have antagonized TGF-β signaling [129] possibly through the modulation of either the up or downstream mediators of TGF-β signaling. Huang *et al.* [123] have demonstrated that upregulation of miR-23a approximately 27a approximately 24 in early stage of human hepatocarcinogenesis attenuates TGF-β-induced tumor suppression. Also, by a feedback inhibition mechanism miR-127 was shown to disrupt TGF-β/c-jun-induced HCC cell migration via MMP13 [130]. Similarly, miR-183 inhibited TGF-β_1-induced apoptosis by down-regulating PDCD4 expression in human HCC cells [48]. Further TGF-β_1-mediated upregulation of hepatic miR-181b was reported to promote hepatocarcinogenesis by targeting TIMP3 [100]. Many other microRNA clusters including miR-106b-25/miR-17-92 have been shown to modulate TGF-β signaling in hepatocarcinogenesis via multiple mechanisms [124]. TGF-β induces the expression of miR-216a and miR-217 which in turn activate PI3K/Akt pathway and through this pathway miR-216a and miR-217 participate in a TGF-β-mediated fibrogenesis and survival of tumor cells [131].

Additionally, TGF-β signaling in late HCC normally is negatively modulated by inhibitory Smad7 through a negative feedback mechanism but a number of studies using TGF-β- stimulated cells have shown decreased expression of Smad7 [132-134]. miR-21 which was overexpressed after TGF-β stimulation was shown to have down-regulated Smad7 expression to enhance TGF-β activity in HIV-infected human liver tissues [64]. MicroRNAs can also interact with signaling proteins of TGF-β specifically the Smad proteins.

INTRICATE INTERACTIONS BETWEEN MICRORNAs AND THE SMAD PROTEINS IN HEPATOCARCINOGENESIS

Smad proteins which mediate transcriptional responses of TGF-β have been observed to interact with the microRNA processing machinery, specifically DROSHA, DGCR8 and p68. For instance TGF-β_1 facilitates DROSHA/DGCR8/p68/Smad interaction to increase the processing of pre- and mature miR-21 expression [10]. By using co-immuno-precipitation and immuno-precipitation a number of studies have shown that Smads complex with DROSHA and p68 on pri-miR-21 hairpin after TGF-β_1 stimulation [1]. Additionally, DROSHA binding to the pri-miR-21 was reported to

increase following ligand stimulation, an observation highly suggestive of the fact that Smads may promote DROSHA-microRNA hairpin binding [10]. Interestingly, interaction between the R-Smads (Smad2 and Smad3) and DROSHA processing complex did not involve Smad4 [10]. Hitherto, the understanding has been that Smad2/3 and Smad 4 complex translocate into the nucleus together as a complex [135]. But recent findings seem to indicate that both Smad2/3 and Smad4 are independently translocated into the nucleus via different import mechanisms [136]. It is probable that monomeric R-Smads (Smad2 and Smad3) which are not bound to Smad4 may participate in microRNA processing by associating with DROSHA-DGCR8 processing complex independently of canonical TGF-β/Smad signaling [10].

Recently, Smad nuclear interacting protein 1 (SNIP1) was detected in DROSHA complex [137] and it is speculated that it could promote efficient processing of pri-microRNA to pre-microRNA of many oncomirs as well as microRNAs that function as tumor suppressor genes. Wang *et al.* [10] have indicated that SNIPI could possibly participate in microRNA biogenesis by interacting with Smad2/3 to promote the function of the DROSHA processing machinery. It is further explained that down-regulation of SNIP1 in mammalian cells could possibly reduce the expression of a subset of microRNAs including oncogenic miR-21 [137]. To verify whether monomeric Smad2/3 may be involved in microRNA biogenesis independently of TGF-β stimulation, a number of studies have used silencing of Smad2/3 through knockout to confirm or otherwise. To this end, a study involving Smad2/3 knockout mice, up-regulation of mature and pre-miR-21 following TGF-β stimulation was blocked, but there was no change in pri-miR-21 transcription [1], suggesting the possible modulation of mature and pre-miR-21 by Smad2/3-dependent signaling. Further, Smad2/3 has been identified as binding partners of p68, an observation which add to the idea that the Smad2/3 could associate with the DROSHA complex [138]. Specifically, Davis *et al.* [1] have shown that the interaction between p68 and Smad2/3 was C-terminal dependent and that the interaction was not affected by RNaseA. This perhaps indicates that p68-Smad2/3 interaction may not affect pri-microRNAs as observed previously but that it may affect pre-microRNA and mature microRNA processing. However, interaction between Smad2/3 and DROSHA was shown to be significantly reduced by RNaseA, implying pri-microRNAs and Smad2/3/DROSHA association may be enhanced by pri-microRNA transcripts [1]. The common Smad (Smad4) inversely related to miR-224 up-regulation and it was shown that Smad4 down-regulation significantly correlated with poor survival of HCC patients [139, 140]. Nuclear accumulation of Smad2/3 and Smad4 is critical for microRNA biogenesis. This preferential nuclear accumulation may be enhanced by mitogenic phosphorylation of the linker region of Smad2/3 by the MAPKs. How then does the MAPK pathway affect the processing and expression of microRNAs?

INTRICATE INTERACTIONS BETWEEN MICRORNAs AND THE MAPKs PATHWAY IN HEPATOCARCINO-GENESIS

The MAPKs modulate mitogenic signals via phosphorylation of the linker region of Smad2/3, and this increases nuclear localization of Smad2/3. Smad2/3 promotes binding and stability of DROSHA/pri-microRNA association, but this process as stated earlier occurs in the nucleus. Translocation of Smad2/3 into the nucleus is therefore crucial for DROSHA-pri-microRNA binding. The nucleo-cytoplasmic shuttling between the nucleus and the cytoplasm by inactive (Non-phosphorylated) Smad2/3 and also the preferential nuclear accumulation of their phosphorylated forms (pSmad2/3) is partly the preserve of canonical TGF-β signaling through formation of Smad2/3/4 complex and their subsequent nuclear translocation. However, the MAPKs have also been shown to increase the nuclear accumulation of Smad2/3 through linker-specific phosphorylation of Smad2/3 [141]. It is quite reasonable that Smad-dependent regulation of the biogenesis of microRNAs could be modulated quiet independently of TGF-β_1 and BMPs by mitogenic signals generated by the MAPKs which increase nuclear localization of Smad2/3. This suspicion is reinforced by the fact that Smad4 appears not to be involved in TGF-β-dependent modulation of microRNA biogenesis.

In fact, a number of microRNAs target the MAPK pathways and their downstream signaling molecules to exhibit both oncogenic and tumor suppressive effects in hepatocarcinogenesis. For example, miR-21 suppresses PTEN and hSulf-1 expressions in HCC, thereby promoting HCC progression via activation of Akt/ERK pathways [110]. In a similar fashion, miR17-5p was shown to promote migration of human HCC cells through activation of p38/heat shock protein 27 pathways [142]. Recently, Song *et al.* [143] have demonstrated that polycyclic aromatic hydrocarbon (PAH)-induced up-regulation of hepatic miR-181 promoted HCC cell migration by targeting MAPK phosphatase 5, a p38-specific activator.

Another indication of corporation between TGF-β and the MAPKs on the biogenesis of microRNAs is shown through miR-127. Overexpression of miR-127 was shown to suppress cell migration, invasion and tumor growth in HCC cells [130]. Meanwhile, miR-127 was shown to directly inhibit TGF-β-mediated activation of matrix metalloproteinase 13 (MMP13 or Collagenase-3) [130]. But TGF-β_1 activates ERK and JNK, which in turn inhibit miR-127 through inhibition of the p53 pathway [130]. Sustained activation of JNK is crucial for the progression of HCC [144, 145] possibly through down-regulation of $p21^{CIP1}$ expression but up-regulation of c-Myc [144]. MiR-21 has severally been shown to be overexpressed in HCC [23, 32, 146], meanwhile, PTEN and Sprouty, two validated targets of miR-21 in HCC [147] were reported to have remained unaltered in liver-specific JNK-deficient mice at least in their mRNA form [148]. Whiles JNK-deficiency in mice had no effect on the protein expression of PTEN; it however increased the protein expression of Sprouty [148], possibly suggesting that miR-21 might have down-regulated Sprouty via JNK. A further test of the functional relevance of increased Sprouty showed reduced Akt and ERK activities, perhaps adding credence to the suspicion that miR-21 just as other oncomirs overexpressed in HCC disrupt their targets by using the MAPK pathway as a decoy. Interestingly, in late stages of carcinogenesis Smad-independent TGF-β-induced EMT was reported to be mediated through activation of ERK [149].

TGF-β activates JNK and p38 through MKK4 and MKK3/6 respectively [150] in many cell lines [151, 152] but PDCD4, a target of miR-21, negatively modulates the activation of c-jun/JNK via suppression of MKK4 (An upstream protein of the MAPK pathway). It is tempting to speculate that oncomirs that are overexpressed in hepatocarcinogenesis modulate the TGF-β, Ras/PI3K, and the MAPK pathways as a decoy to repress the expression of important tumor suppressor genes (PDCD4, Sprouty, Smad7, and PTEN) in order to promote progression of hepatocarcinogenesis as illustrated in (Fig. **1**). A careful disruption of this signaling network might offer new targets and leads for therapeutic interventions against hepatocarcinogenesis.

THERAPEUTIC POTENTIALS OF MICRORNAs

MicroRNAs may function as tumor suppressors genes or oncogenes [155]. As a result, they may affect the etiology, diagnosis and prognosis of many cancers [156]. More than 50% of profiled microRNAs are reported to be located on cancer-linked genomic loci [155-157], therefore provide a fine platform for identification and tracing the origin of hitherto cancers with poor prognosis. MicroRNA therapeutics which embody strategies to silence oncomir biogenesis and function or restore microRNAs that function as tumor suppressor genes to augment existing cancer-specific therapies derives from the ability of microRNAs to target many protein coding genes, most of which partake in cancer. In the ensuing paragraphs, we make some few suggestions on the value of liver-specific microRNAs as biomarkers, staging tools and also possible strategies to silence oncomirs or restore tumor suppressive microRNAs. Lu *et al.* [11] have posited that microRNA profiling and signatures analyzed in a number of human cancers seem to provide a much better accuracy in tumor diagnosis and identification than even mRNAs. Subsequently, microRNA signatures were shown to be well suited for diagnosis and also grouping human cancers into subtypes [14, 158, 159].

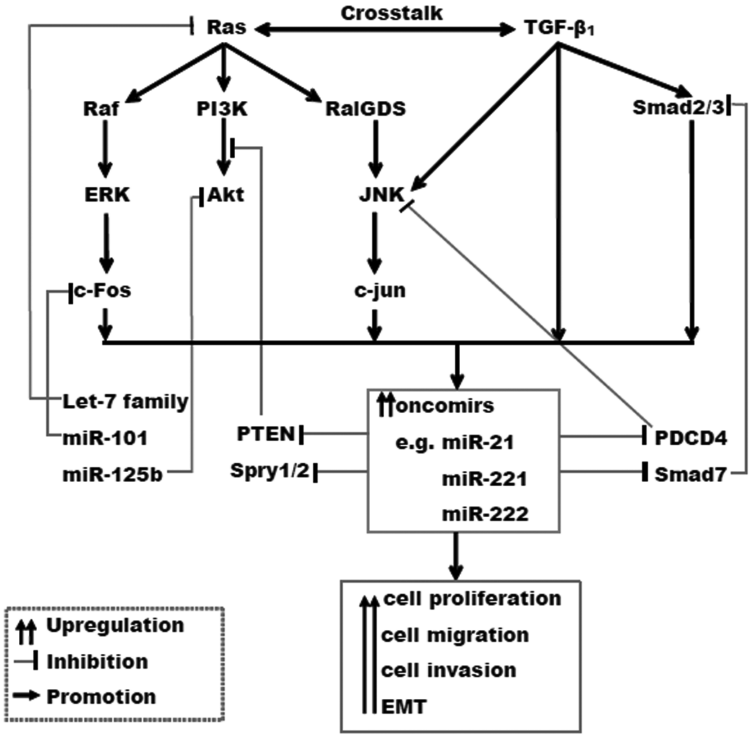

Fig. (1). The illustration represents a summary from the text relating the possible intricate conspiracy existing between TGF-β_1/Smad, Ras/PI3K/Akt and the MAPK pathways and their combined functional output on the expression of microRNAs overexpressed in hepatocarcinogenesis (Oncomirs, e.g. microR-21, miR-221 and miR-222), some important tumor suppressor genes (PTEN, Sprouty, PDCD4 and Smad7), microRNAs functioning as tumor suppressors (Let-7 family, miR-101 and miR-125b) and some phenotypic hallmarks of hepatocarcinogenesis. Dysregulated TGF-β/Smad signaling in late hepatocellular carcinoma (HCC) is normally subverted by tumor cells to their advantage, thereby turning the tumor suppressor function of TGF-β_1 into tumor promotion. As a result, both canonical TGF-β_1 signaling through Smad2/3/4 and non-canonical TGF-β_1 signaling through crosstalk with the MAPK and Ras/PI3K/Akt pathways facilitate the biogenesis of oncomirs such as microR-21. Meanwhile microR-21 overexpression represses the expression of inhibitory Smad7 [64], PTEN [23, 153], PDCD4 [46] and Sprouty1/2 [154] leading to manifestation of phenotypic hallmarks of hepatocarcinogenesis. But the tumor suppressor genes together with Let-7 family of microRNAs, miR-101, and miR-125b negatively modulated the TGF-β, MAPK and Ras/PI3K/Akt signaling pathways. MiR-221 and miR-222 repress PTEN [154], miR-101 targets c-Fos [43], Let-7 family represses Ras-Raf-Mek-ERK cascade [26]. Abbreviations: EMT (Epithelial to mesenchymal transition), ERK (Extracellular signal-regulated kinase), PI3K (Phosphatidylinositol-3-kinase), TGF-β (Transforming growth factor β), MAPK (Mitogen activated protein kinase), PTEN (Phosphatase and tensin homolog), JNK (c-jun N-terminal kinases), PDCD4 (Programmed Cell Death Protein 4). The bold arrowed lines show the oncogenic pathways and the cross signaling between TGF-β_1, Ras, and JNK leading to expression of oncomirs while the thin and faint lines with blunt ends show the tumor suppressive network.

In agreement with the many expert views, liver-specific microRNA expression patterns in hepatocarcinogenesis could be characterized into hepatic microRNA signatures for use as biomarkers for early liver cancer risk assessment. As it is globally acknowledged, in the fight against cancer early detection is important. In this sense, liver-specific microRNA expression patterns could be used as a basis for staging hepatocarcinogenesis or as a means to categorize liver cancer in general into subtypes. For example, which set of liver-specific microRNAs are aberrantly expressed as liver disease progresses from fibrosis to cirrhosis and finally to HCC? And how does the expression pattern of hepatic microRNAs relate to the expression of known oncogenes or tumor suppressors genes? Already some works have started serotyping liver cancer into subtypes based on microRNA signatures. Several independent studies [23, 25, 146, 160] have reported the overexpression of microR-21 in HCC. These convergent reports further reinforce the long held view of micrR-21 as a complete oncogene in a number of human cancers. Aside the use of liver-specific microRNA signatures as diagnostic tools, those identified as oncomirs could serve as targets for therapy. For instance, oncomirs could be silenced by targeted depletion of their specific precursors. Notably, pri- and pre- transcripts of liver-specific oncomirs could be targets for oligonucleotide- and small molecule-based strategies to abrogate their oncogenic roles in hepatocarcinogenesis. On the other hand, microRNAs that function as tumor suppressors could be restored using their mimics. MicroRNA therapeutic approach if well characterized by overcoming underlying challenges could offer an important avenue for therapy against specific liver cancers. For example, depletion of miR-21-specific SNIP1 could abrogate TGF-β_1/miR-21-dependent oncogenic activity in late HCC. In much the same way, down-regulation of p68 or blockade of receptor-specific Smads (Smad1, Smad2, Smad3 and Smad5) could repress the expression of miR-21 and other oncomirs implicated in hepatocarcinogenesis. This is because the Smads as has been explained earlier facilitate the biogenesis of a number of oncomirs implicated in many human cancers. Therefore it is important to consider all the processes leading to the biogenesis of microRNAs as potential targets for therapy.

Recent insights into microRNA biology seem to indicate that after microRNA biogenesis from resident cells, they are leaked into circulation to be disseminated to distant sites in the event of inflammation or tissue injury. Subsequent investigations have revealed that microRNAs are package into microvesicles and exosomes then later exocytosed into extracellular environment to exert paracrine and hormone-like functions either in the short or long term. How can this mechanism of microRNA storage and release be manipulated to yield therapeutic gains? It is reasonable to think of microRNAs as having specific membrane-bound receptors which probably facilitate their target cell recognition and their colonization at the new sites. Indeed, the stable presence of microRNAs in body fluids could be used for diagnostic purposes. Also strategies could be designed to disrupt the storage and release of specific microRNAs, particularly the oncomirs to abrogate their oncogenic roles in hepatocarcinogenesis. This may probably help to reduce the bioavailability of oncomirs in circulation or at least keep their expression outputs well below functional levels. Also, it is worth suggesting perhaps the complete blockade of membrane-bound receptors of oncomirs provided the blockade will be specific to only the oncomirs of interest. Identification of a whole therapeutic class of either pharmacologically active agents or small molecules that can disrupt oncomirs at the precursor and receptor level could well be the focus of future investigations.

Further, microRNA replacement therapy using small molecules has gained quiet substantial investigative attention and probably holds promise for the treatment of hepatocarcinogenesis. For instance, hypomethylating agents (Decitabine and 5-azacytidine) were reported to have improved the expression of silenced tumor suppressive microRNAs and as a result they have been considered for the approval of the treatment of myelodysplastic syndromes [161]. It is hoped that many target-specific small molecules in the likes of the hypomethylating agents could be discovered in the future to be used in restoring the expression of silenced microRNAs that function as tumor suppressors in hepatocarcinogenesis. Recently the interest has drawn towards the use of oligonucleotide-based approaches to either restore expression of silenced tumor suppressive microRNAs or repression of oncomirs. For example, miR-145 has been reported to be repressed in HCC but its exact targets are yet to be determined. Due to the consistent downregulation of miR-145 in HCC, it could be said that it functions as a tumor suppressor in HCC. As a result, miR-145 expression can be restored in HCC patients by using microRNA mimics or oligonucleotide-based approaches.

Additionally, many microRNA inhibitory approaches including antisense oligonucleotides (ASOs) such as locked nucleic acids (LNA anti-mirs), tiny LNA anti-mirs, antagomirs and microRNA sponges as reviewed in Ling *et al.* [73] are reported to be at various stages of clinical investigation as a part of new microRNA therapeutics. In the same direction, microRNA expression vectors bioengineered with promoters for microRNAs of interest could be used as as a part of the therapeutic machinery for specific liver cancers. A typical example is miR-26a which is normally silenced in HCC, but using the above strategy Ji *et al.* [162] have demonstrated in both *in vitro* and xenograft mouse models of HCC the restoration of miR-26a expression leading to abrogation of tumor progression. Still with miR26a, a bioengineered miR-26a expression vector with a dual promoter for α -fetoprotein and human telomerase reverse transcriptase was reported to have restored miR-26a expression in HCC leading to abrogation of HCC progression via down-regulation of estrogen receptor-α and inhibition of cyclin-dependent and independent pathways [163]. Refreshingly, microRNA replacement therapies are yielding positive results with specific reference to liver cancer. A noteworthy example is the recently synthesized MRX34, a liposome-formulated mimic of tumor suppressor miR-34, which has been shown to produce complete tumor regression in two mouse models of liver cancer [73]. As a result, it is been conducted into a phase 1 clinical trial for possible enrollment into therapies against HCC. It is envisioned that in the not too distant future many microRNA therapeutic strategies (Fig. **2**) will be enrolled into the existing therapies

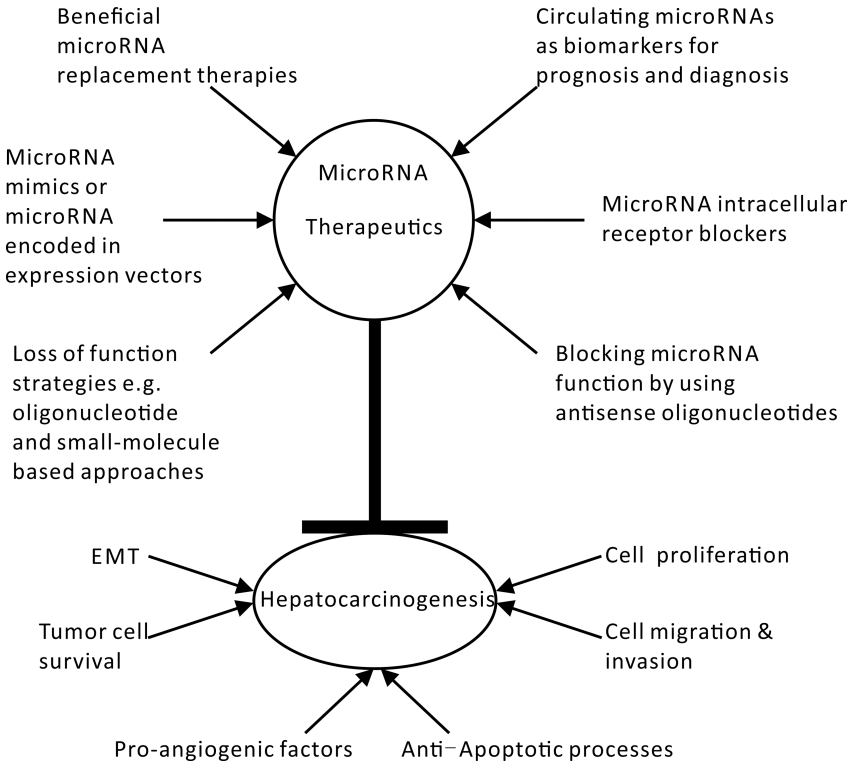

Fig. (2). A summary of microRNA-based therapeutic approaches for possible use in the treatment of hepatocarcinogenesis, EMT (Endothelial-mesenchymal transition).

to enhance the treatment of hepatocarcinogenesis and also to improve recovery and survival of HCC patients.

CHALLENGES OF MICRORNA RESEARCH/THERAPY

First, the heterogeneity both in sampling and methods used in microRNA investigations are the sources of many contradictory reports. For instance, many of the inconsistent reports on microRNA profiling could be traced to the different methods used by different research scientists in different geographical locations. It is necessary that a standard protocol is agreed upon and designed by experts in the field for use by all research scientists irrespective of one's geographical location. This at least will provide some level of uniformity and probably decrease the level of contradictory reports.

Next, microRNA therapeutics must be designed in such a way to overcome the problem of non-specific targeting and off-target effects commonly associated with chemotherapy. As an example, a single microRNA may have a number of mRNA targets including oncogenes and tumor suppressor genes in one cell type. Compounding an already difficult situation is the fact that some specific microRNAs can functionally behave like oncogenes in one cell type and as a tumor suppressor in another cell. For instance, overexpression of microR-221 in liver cancer exerts oncogenic phenotypic consequences partly through downregulation of PTEN [118], however in erythroblastic leukaemia, the same microR-221 acts as a tumor suppressor gene by repressing the expression of the KIT oncogene [164]. The difficulty lies in how to manipulate a particular microRNA signature in one cell type or cancer subtype to achieve a therapeutic objective without risking undue detrimental phenotypic consequences in other cell types. So there is a need to consider lessening the likely activation of compensatory oncogenic pathways, which in most instances serve as sources of undue side effects. MicroRNAs therapeutics must therefore be carefully and exhaustively designed to suit a specific therapeutic need.

Another point is that the delivery of microRNAs of interest and other microRNA constructs to target sites has always been a major challenge. But fortunately the emergence of nanoparticle technology, it is hoped that microRNA therapeutics will be designed to ensure effective delivery at the target cells in a more stable form. In light of this statement, pegylation in liposomes, use of monoclonal antibodies and other delivery constructs are of paramount importance. Even more refreshing is how macrophages can now be reached by many mechanisms, including delivery through constructs to mannose receptor [165]; scavenger receptor [166]; folate receptor [167] and through high molecular weight compounds such as liposomes, polymers or mycelles [168].

FUTURE EXPECTATIONS

The discovery of microRNAs and their subsequent implication in disease pathogenesis offers a fine opportunity for prognosis, diagnosis and treatment of many diseases including cancer. Particularly, it is expected that a well characterized multi-target yet specific and effective individualized microRNA therapeutics that suit not only

patient peculiar presentations but also the cancer subtype will be enrolled into the existing conventional anti-cancer therapies as replacements, adjuncts or as a part of combinatorial formulations for the treatment and management of cancer in general. Indeed, the microRNA revolution in therapeutics offers an unmatched opportunity in the war against the global cancer threat.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIALS

Supplementary material is available on the publisher's web site along with the published article.

LIST OF ABBREVIATIONS

AP-1 = Activator protein-1

ASO = Antisense oligonucleotides

CDK = Cyclin-dependent kinase

CDK4 = Cyclin-dependent kinase 4

DGCR8 = DiGeorge syndrome critical region 8

DNA = Deoxyribonucleic acid

ECM = Extracellular matrix

ERK = Extracellular signal-regulated kinase

HBeAg = Hepatitis B e antigen

HCC = Hepatocellular carcinoma

HDAC4 = Histone deacetylases 4

HSC = Hepatic stellate cell

hSulf-1 = Human sulfatase 1

IFNγ = Interferon γ

IL = Interleukin

iNOS = Inducible nitric oxide synthase

JNK = c-jun N-terminal kinases

LNA = Locked nucleic acids

MAPK = Mitogen activated protein kinase

MMP = Matrix metalloproteinase

mRNA = Ribonucleic acid

PAF = Platelet activating factor

PDCD4 = Programmed Cell Death Protein 4

PI3K = Phosphatidylinositol 3 kinase

PTEN = Phosphatase and tensin homolog

RISC = RNA-induced silencing complex

STAT3 = Signal transducer and activator of transcription 3

TIMP-1 = Tissue inhibitor of metalloproteinase-1

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