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The Duality of OncomiR Addiction in the Maintenance and Treatment of Cancer

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Abstract

It has long been established that cancers can become addicted to particular oncogenes. Despite the genetic complexity that governs tumorigenesis, certain cancers can exhibit a critical dependency on the expression of a single oncogene, which when removed leads to death of the cancer cell. Recent observations on the relationships between regulatory RNAs and cancer have revealed that this concept of oncogene addiction extends to microRNAs (miRNAs) as well. Certain cancers exhibit a dependency on the expression of a single oncogenic miRNA, or oncomiR. The field of miRNA biology and its involvement in diseases such as cancer has seen tremendous advances over the past decade. However, little is known about the phenomenon of oncomiR addiction. In this review, we introduce the concept of proto-oncomiRs, or miRNAs that gain oncogenic activity after an initiating event. Further, by highlighting the role of proto-oncomiRs in generating malignant phenotypes, we glean possible insights into the mechanisms that guide oncomiR addiction. Additionally, toward the realization of genetically-driven personalized medicine, some of the most clinically successful anticancer strategies have involved targeting addictive oncogenes such as HER2, BCR/ABL, EGFR, and VEGF. Elucidating how addictive miRNAs can perpetuate cancer may reveal additional critical molecular targets to exploit for therapeutic purposes. Therefore, in this review, we also summarize the field of anti-miRNA therapeutics, in which antisense and nanoscale delivery technologies are the driving force. Addictive oncomiRs are a double-edged sword; addicted cancers are dependent on oncomiRs that are highly potent therapeutic targets. Dissection of this phenomenon may reveal the mechanisms through which lynchpin miRNAs can perpetuate cancer and present a new paradigm for miRNA-based cancer therapy.

Keywords

oncomiR addiction; oncogene; microRNA; proto-oncomiR; tumorigenesis; oncogenesis; cell circuitry; anticancer therapy; anti-miR; molecular targeting

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Oncogenic MicroRNAs and Cancer

Endogenously expressed in most all cells and tissues, microRNAs (miRNAs) comprise a class of non-coding single-stranded RNAs that facilitate gene downregulation by both translational repression and mRNA destabilization mechanisms (1). Bioinformatic estimates predict that at least 30% of all of the human genes are regulated by miRNAs (2). Owing to their ubiquitous expression patterns and functional diversity, miRNAs play key roles in many biological processes; including gene regulation, cell developmental control, and the development of various diseases, such as cancer (3). Dysregulated miRNA expression has been described in many types of cancer (4). In 2002, the Croce group reported the first link between miRNA and the pathogenesis of cancer; specifically, miR-15a and miR-16-1 were observed to be downregulated in B-cell chronic lymphocytic leukemia patients (5). It is now appreciated that miRNA expression signatures of human tumors can be used to classify cancers (4). Soon after, many of the known miRNA genes were shown to reside in genomic regions that are commonly associated with cancer (6). In fact, miRNAs themselves are now known to act as tumor suppressors and oncogenes.

OncomiRs are miRNA oncogenes that have a causal role in the onset and/or maintenance of cancer phenotypes. Cancer represents the summation of a myriad of genetic and epigenetic abnormalities found in genes involved in diverse biological pathways (7). Despite this complexity, cancers can be dependent on a single oncogene; such that abrogation of this oncogene leads to loss or reversion of the malignant phenotype. This phenomenon is perhaps most well-described for the MYC oncogene, in which overexpression of MYC in a variety of cells can promote tumorigenesis, and inactivation of MYC is sufficient to reverse the induced malignancy (8). Oncogene addiction—introduced by Weinstein et al. (9)—relies on proteins with diverse functions, such as transcription factors (i.e. MYC), growth factors, signaling kinases, and regulatory GTPases. Theories for why an oncogene could be addictive include the possibility that increased negative feedback inhibition is active in cancer cells to control for the active oncogene, so that when the oncogene is turned off, this leads to excess inhibitory signals and cell death. Alternatively, the oncogene may take over for normal gene functions, so that when it is inhibited, cancer is suppressed by lack of compensatory normal gene function or through a return to a phenotypically normal state (10). The elucidation of integral relationships between miRNAs and cancer further extends this ‘addiction’ model to regulatory miRNAs, in a concept termed oncomiR addiction.

Of all the oncomiRs identified thus far (Table 1), miR-21 has been shown to have elevated expression levels in the most diverse subset of cancerous cell lines and tissues, including glioblastoma, breast, colorectal, lung, pancreas, skin, liver, gastric, cervical, thyroid, and various lymphatic and hematopoietic cancers (4,11). In accordance with its abundance in many cancers, miR-21 is a potent oncomiR. Several *in vitro* and *in vivo* models have demonstrated that ectopic expression of miR-21 can be associated with tumorigenesis and cancer progression. Liu et al. found that transfection of prostate cancer cells with pre-miR-21 promoted angiogenesis (12). Asangani et al. linked increased invasion, intravasation, and metastases to the overexpression of miR-21 in colorectal cancer cells (13). Both Hatley et al. and Frezzetti et al. demonstrated that when associated with KRAS oncogene activation, induction of miR-21 led to neoplastic transformation in lung cancer mouse models (14,15). Also, Medina et al. reported that direct and conditional overexpression of miR-21 in transgenic mice was sufficient to induce an aggressive pre-B cell lymphoma phenotype (16). Common amongst all of these studies is that the miR-21-initiated malignancy can be halted, or even reversed, with the depletion or knockout of miR-21. In the first example of oncomiR addiction, the Medina study demonstrated that direct (i.e. Tet-off system) shut down of the miR-21 oncogenic ‘switch’ led to cancer

regression. Together, these studies highlight the integral role that a single miRNA can have in the initiation, maintenance, and survival of oncomiR-addicted cancers.

Causes of OncomiR Addiction

The complex molecular networks through which miRNAs can promote carcinogenesis have been well-studied (17,18). However, it remains unclear why cancers can become addicted to the expression of a single oncomiR. The need for continued oncomiR activity to maintain the cancer phenotype may depend on many factors, such as its expression levels, the spatio-temporal context, the downstream functions of the oncomiR-suppressed targets and pathways, as well as external influences (Figure 1).

Expression levels

OncomiRs are characterized by high expression levels in tumors relative to normal tissues. MicroRNA profiling studies typically require at least a 2-fold increase in expression for the miRNA to be considered elevated (4,19). In independent studies in which the induction of either miR-21 or miR-155 in transgenic mice resulted in lymphoid malignancy, the miRNAs were expressed at levels up to 20-fold greater than in wild type tissues (16,20). In certain forms of B cell lymphoma, the levels of miR-155 (~8,500-10,000 copies per cell) are increased up to 60-fold relative to control B cells (21). Additionally, in some cancers, miR-21 occupies 15-25% of the total cellular miRNA levels (11). Clearly, high expression of oncomiRs can be correlated with malignancy; however, it is unknown whether there is a miRNA expression threshold above which, cancers can become addicted to an oncomiR. Development of mouse models with control over the degree of oncomiR overexpression would enable investigation of this possible threshold effect. Nonetheless, such a threshold would likely be specific to the type of cancer and associated oncomiR. Tili and Croce maintain that while miR-155 overexpression can lead to lymphomagenesis, the expression levels in these tissues are intermediate compared to the up-regulated levels of miR-155 in other cellular pathways, such as inflammation (22). Therefore, given the involvement of cancer-associated miRNAs in biological processes unrelated to cancer, it is likely that other factors beside expression level contribute to the onset of addiction.

Spatio-Temporal Context

While oncomiRs typically have high expression levels in associated cancers, most cancer-associated miRNAs are also differentially expressed throughout non-cancerous cells and tissues. OncomiRs typically do not cause cancer in every cell in which they reside. The cellular context of cancer-associated miRNAs can have a drastic impact on function. Several miRNAs, such as miR-125b, miR-29, miR-143, and miR-146, have been described as functioning as tumor suppressors or oncogenes depending on the cell type, among other factors (23-28). Therefore, it is likely that the development of addiction is also dependent on cell and tissue type. Although miR-155 is expressed from an evolutionarily conserved region of the hematopoietic enriched *BIC* gene, the ratio of BIC transcript levels to miR-155 varies with cell type (22). In some contexts, miR-155 is involved in hematopoiesis and the immune response; however, miR-155 also has an oncogenic role associated with different types of lymphoma (29). The levels of miR-155 even varies amongst various lymphoid cancers: high levels of miR-155 are present in diffuse large B cell lymphoma, B cell chronic lymphocytic leukemia, Hodgkin lymphoma, and primary mediastinal B cell lymphoma; however, low levels are typically detected in non-Hodgkin lymphomas (21). In a related unpublished study on miR-155, we observed that ectopic overexpression (under control of the nestin promoter) of miR-155 in lymph tissue of transgenic mice drove the pathogenesis of disseminated lymphoma; on the contrary, similar overexpression of miR-155 in brain and lung tissue was insufficient to promote tumorigenesis (submitted). Ubiquitously expressed miR-21 also

displays tissue-specific oncogenicity; despite being associated with a multitude of cancers, Folini et al. observed that the oncogenic properties of miR-21 are not central to the development of prostate cancer (30). The cellular setting appears to be essential for defining the roles of miRNAs in cancer.

Temporal factors may also contribute to the onset of addiction. Costinean et al. observed that induction of miR-155 results in early pre-B cell leukemia and lymphoma followed by malignant B cell transformation at later life stages (20). This delayed malignancy was a result, in part, due to continued overexpression of miR-155. Furthermore, short duration of expression is thought to contribute to the inability of miR-155 to induce cancer when overexpressed in lymphocytes during immune response activation (22). The acute regression of oncomiR-driven cancer models in response to miRNA withdrawal suggests that constitutive overexpression of miRNAs may be also essential to foster a dependency on specific oncomiRs.

Down-Regulated Targets

A key consideration concerning the dependency of cancer on the expression of specific oncomiRs involves the targets that are attenuated by the miRNAs. OncomiRs perpetuate cancer through regulation of biological pathways that include apoptosis, proliferation, differentiation, cell senescence, and angiogenesis. Within these processes, a multitude of tumor suppressor targets have been identified in models of oncomiR-induced cancer (17,31,32). Despite the vast miRNA regulatory networks that have been elucidated in cancer, it remains unclear which of these targets, if any, are effectors leading to oncomiR addiction. On the contrary, certain miRNAs exhibit oncogenic behavior through association with virus-induced cancers. In liver cancer, both miR-122 and miR-141 have been shown to sustain HCV infection, which can lead to hepatocarcinogenesis (33,34). In this setting, hepatocarcinoma is addicted to oncomiRs via the activities of miRNA-regulated HCV.

Feedback loops exist between miRNAs and their targets, in which the expression or biogenesis of miRNAs can be controlled by transcription factors that are directly or indirectly regulated by the miRNAs themselves (35-39). The oncogenic miR-17~92 cluster has been shown to be involved in a multi-level negative feedback loop involving E2F family of cell cycle and apoptosis controllers, as well as c-MYC. O'Donnell et al. and Sylvestre et al. observed that the oncomiR, miR-20a (a member of the miR-17~92 cluster), down-regulates E2F1, E2F2, and E2F3, and E2F1-3 binds to the promoter of miR-17~92 to activate transcription of the cluster (40,41). Furthermore, the E2F family initiates the expression of its own genes in positive auto-regulatory loop (42). MYC adds another layer of complexity through its transcriptional activation of miR-17~92 and the positive feedback loop between MYC and the E2F family (41). Ultimately, this regulatory network contributes to the oncogenicity of miR-17~92, and correction of deregulated miR-17~92 expression leads to reversion of malignancy (43). Given the involvement of miRNAs in various regulatory circuits, it is likely that feedback loops may play a role in potentiating oncomiR addiction.

External Influences

Similar to proto-oncogenes, some miRNAs require a 'primum movens,' or initiating event, before they can function as oncomiRs—in a sense, they can be considered proto-oncomiRs. As previously mentioned, in miR-21-overexpressing mice crossed with the *Kras*^{LA2} spontaneous murine lung cancer model, Hatley et al. saw that miR-21 significantly enhanced tumor formation in cooperation with activated KRAS (15). Interestingly, they observed that ubiquitous expression of miR-21 alone (4- to 6-fold increase relative to wild type) was insufficient to drive tumorigenesis. Using a transplantable tumor model, Swarbrick et al.

observed that forced overexpression of miR-380-5p promoted mammary tumorigenesis in cells with activated HRAS (44). Additionally, He et al. demonstrated that when overexpressed, the miR-17~92 cluster cooperated with c-MYC to potentiate tumorigenesis in B cell lymphoma (45). Furthermore, certain viruses function as external oncomiR switches. In Adult T Cell Leukemia cells that had been transformed by the HTLV-1 retrovirus, cancer was initiated by the virus-encoded Tax oncogene, yet is thought to be maintained by deregulated miRNA expression (46). Also, B cell infection by the Epstein-Barr virus induces expression a multitude of miRNAs, including miR-155, which is involved in subsequent cell immortalization (47,48). Some miRNAs are even encoded by oncogenic viruses; Kaposi's sarcoma-associated herpesvirus expresses its own homologue of miR-155, miR-K12-11, which mimics some of the regulatory functions of miR-155 (49,50). Similar to this interplay of miRNAs with protein and virus effectors, it is likely that oncomiR addiction could result from the activities of external factors.

In a sense, miRNAs can be considered proto-oncomiRs until triggered to function as oncogenes. Further elucidation of the biological conditions and molecular circuitry governing oncomiR-driven cancers is required to ascertain if such events are required for initiating oncomiR addiction.

Strategies to Target OncomiR Addiction for Cancer Therapy

OncomiR-addicted cancers present a promising target for anti-miRNA-driven therapy (Figure 1). The therapeutic impact of oncomiR inhibition is well-documented, and has been successfully achieved using a variety of different methods.

Regulatory Elements

Transcriptional control of oncomiR expression is a viable therapeutic strategy. Chang et al. recently reported that BRCA1 facilitates deacetylation of histones on the miR-155 promoter (51). Therefore, BRCA1 is a tumor suppressor that epigenetically down-regulates miR-155. In diffuse large B cell lymphoma, miR-155 expression is stimulated by the pro-inflammatory cytokine, TNF α . Pederson et al. observed that administration of the protein-based anti-TNF α drugs, etanercept or infliximab, reduced miR-155 levels and delayed growth of B cell xenograft tumors (52). Along these lines, Watashi et al. screened a library of small molecules for suppressive effects on oncogenic miRNAs (53). They identified compounds that were able to inhibit the tumorigenicity of NIH3T3 cells that overexpress the oncomiRs, miR-93 and miR-130b. These molecules functioned through global arrest of miRNA biogenesis, so further refinement toward oncomiR specificity is required. However, this system highlights the therapeutic potential of down-regulating miRNA expression.

Naked Antisense Molecules

Administration of nucleic acids (i.e. anti-miRs) that bind to and sequester mature miRNAs is a potent and highly specific therapeutic strategy. The goal of anti-miR therapy is to antagonize the interaction between a complementary miRNA and its target mRNAs. Anti-miRs often are chemically modified (e.g. phosphorothioate modifications, 2'-O-Me and 2'-fluoro substitutions, locked nucleic acids, peptide nucleic acids, and morpholinos) to enhance stability and binding affinity (54). Also, anti-miRs can be modified such that a single molecule inhibits multiple miRNAs; Lu et al. generated a multivalent anti-miR that successfully inhibited miR-21, miR-155, and miR-17-p and attenuated the growth of cultured breast cancer cells (55). Delivery of anti-miRs using commercially available transfection agents has become a common practice for blocking miRNA activity *in vitro*.

Chemically modified anti-miRs have also been used to successfully inhibit oncogenic miRNAs *in vivo*. In one of the pioneering studies demonstrating the utility of anti-miRs *in*

vivo, Krutzfeldt et al. used cholesterol-conjugated oligonucleotides that had been further enhanced with 2'-O-Me and phosphorothioate modifications. The cholesterol moiety functioned to improve the pharmacokinetics and, possibly, the cell uptake of these 'antagomiRs' (56,57), while the backbone modifications increased the stability and binding affinity for miRNA. Inspired by cholesterol-conjugated siRNAs, antagomiRs were effective at inhibiting liver-specific miR-122 after intravenous administration (56) and have been used in a variety of cancer models. Ma et al. showed that delivery of antagomiR against miR-10b prevented metastases in a mammary tumor model (58). In the first report of diminished primary tumor growth in response to locally administered anti-miR treatment, Fontana et al. delivered antagomiRs against miR-17-5p in a neuroblastoma flank tumor model (59).

Other types of chemically modified nucleic acids have also been investigated as anti-miRs *in vivo*. Locked nucleic acid (LNA) oligonucleotides represent one of the most widely used classes of anti-miRs (60,61). Compared to other chemically-modified anti-miRs, LNAs typically have the highest binding affinity with complementary miRNA. Toward the potential treatment of liver cancer, Elmen et al. systemically delivered LNA against miR-122 to suppress HCV viremia in non-human primates (62). In the first report of decreased tumor growth in response to systemically administered anti-miR treatment, Swarbrick et al. delivered fully phosphorothioated, 2'-fluoro- and 2'-methoxyethyl-modified LNA anti-miRs (high dose of 25mg/kg twice weekly for 3 weeks) against miR-380-5p to treat an orthotopic mouse model of neuroblastoma (44). This remains one of the only oncomiR-targeted systemic anti-tumor studies to date. 'Naked' administration of anti-miRs is a promising and clinically achievable strategy for inhibiting miRNA. However, their ability to inhibit cancer-associated miRNAs *in vivo* is potentially hindered by pharmacokinetic hurdles; systemically administered anti-miRs, even LNAs, typically do not actively target to tumors unless given at high doses.

Nanoscale Delivery Systems

One of the primary goals of nanoscale (1-200 nm in size) delivery systems is to safely increase the achievable dose of a drug or anti-miR at the site of interest. Engineered viruses are perhaps the most efficient class of nanoscale vectors; they are extremely proficient at gene delivery (63). Similar to synthetic anti-miRs, viruses can elicit miRNA inhibition through the expression of complementary transcripts that sequester mature miRNAs. Virally-delivered miRNA sponges, miRZip vectors, and tough decoy RNAs have all demonstrated success at inhibiting miRNAs (64-66); however, in a therapeutic setting, these virus-based delivery systems suffer from significant safety concerns such as possible immunogenicity and chromosomal interruptions (67). Non-viral nanoscale systems typically avoid these complications (24). While generally not as efficient as viral systems, non-viral vectors (e.g. lipid nanoparticles, polymer nanoparticles, and dendrimers) provide a safe platform that can be amenable to a diverse array of modifications and delivery enhancements that are particularly suited for cancer therapy. A unique property of synthetic nanoparticles is their ability to target to and accumulate in tumors. This targeting can be achieved via the passive, size-dependent enhanced permeability and retention effect (69), and through coating the nanovehicles with molecules that actively target to tumor cell-specific receptors.

Several nanoscale delivery platforms have been used to inhibit cancer-associated miRNAs. Su et al. systemically administered lipid nanoparticles loaded with 2'-fluoro-modified anti-miRs to inhibit liver-specific miR-122 (70). Liu et al. employed a similar lipid nanoparticle system to deliver anti-miRs against miR-296 to inhibit angiogenesis in a matrigel plug model (71). Using systemically injected liposomes that delivered 2'-O-Me anti-miRs against oncogenic miR-132 (2.5mg/kg every 2 days 8 times), Anand et al. observed tumor growth delay and suppression of angiogenesis in an orthotopic xenograft breast cancer model (72).

Notably, both Anand and Liu coated their nanoparticles with cRGD, a targeting ligand that seeks out the tumor neovasculature via binding to $\alpha_v\beta_3$ integrins. Lastly, in unpublished work, we systemically administered biodegradable polymer nanoparticles encapsulating peptide nucleic acid anti-miRs to inhibit miR-155 in an inducible mouse model of pre-B cell lymphoma (submitted). As with the cRGD-coated liposomes, these nanoparticles were functionalized with a cell-penetrating peptide that improved cellular uptake and therapeutic efficacy. This model was addicted to constitutive expression of miR-155, and as a result, anti-miR-155 treatment was efficacious at a dose (1.5mg/kg twice in 1 week) that was ~50-fold less than the doses used in tumor-targeted antisense LNA oligonucleotide studies. Because of added functionalities imparted by molecules such as cRGD targeting ligands and cell-penetrating peptides, synthetic nanoparticulate systems show great promise for oncomiR-directed therapy.

Conclusion

OncomiRs are powerful molecular tools that modulate a multitude of cancer-associated genes. The multistage and multigene aberrations that drive cancer are intimately linked to miRNAs. Although little is known about the underlying mechanisms, cancers can become addicted to the expression of a single oncomiR, such that withdrawal of the lynchpin miRNA leads to loss or reversal of the cancer phenotype. Factors that contribute to the onset of addiction likely include the level of overexpression, the cell and tissue type, the duration of expression, the activities—including feedback control—of the targets that are regulated by the oncomiR, and other external influences. Further dissection of this phenomenon would provide insights into miRNA and cancer biology, as well as establish critical targets for cancer therapy.

OncomiR-targeted gene therapy has unmet potential as a form of molecular medicine. Strategies to inhibit miRNAs include transcriptional down-regulation as well as the more adaptable method of sequestration of mature miRNAs with complementary nucleic acid-based anti-miRs. Toward the treatment of cancer, the seminal concern that plagues nucleic acid-based therapies is delivery. Naked genetic molecules generally suffer from rapid systemic clearance, instability in the bloodstream, a lack of tumor-specific localization, and inefficient cellular uptake and retention. Chemical modifications have ameliorated some of these concerns; however, the success of miRNA-driven therapeutics in cancer likely hinges on the design of effective delivery vehicles. The use of nanoscale systems to deliver miRNA inhibition is a relatively nascent therapeutic approach. However, recent studies have illustrated their ability to safely overcome physiological and cellular delivery barriers, and inhibit oncogenic miRNAs in malignant tissues. Ultimately, the most efficacious anti-miR-based treatment strategies will likely capitalize on oncomiR addiction and the targeting of molecules that can be described as the ‘Achilles heel’ of cancer.

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Figure 1.

Cancers can be initiated and/or maintained by oncomiRs. The factors that contribute to the onset of oncomiR addiction are unclear, but may include miRNA overexpression, cell/tissue type, duration of miRNA expression, activities of the oncomiR targets, as well as other external influences. Withdrawal of addictive oncomiRs results in suppression of malignancy.

Table 1

Evaluation of oncomiR inhibition in selected cancer models

OncomiR	Cancer Type	Inhibitor	Response to Withdrawal	References
miR-10b	Breast	antagomiR	Reduction of lung metastases [*]	(58)
miR-17~92	Neuroblastoma	antagomiR	Delayed tumor growth	(59)
miR-21	Breast	anti-miR [†] , ex vivo	Delayed tumor growth	(73)
	Breast	PNA, ex vivo	Delayed tumor growth	(74)
	Glioblastoma	LNA [‡] , ex vivo	Delayed tumor growth	(75)
	Lung	Deletion [§]	Delayed tumor growth ^{††}	(15)
	Lung	LNA	Delayed tumor growth	(14)
	Lymphoma	Tet-off ^{**}	Tumor regression	(16)
miR-122	HCC	LNA	Reduction of HCV viremia ^{‡‡}	(62)
miR-132	Breast	2'-O-Me, liposome	Delayed tumor growth	(72)
miR-143	HCC	anti-miR vector ^{§§}	Delayed tumor growth & metastases	(76)
miR-155	DLBCL Lymphoma	Eternacept ^{***}	Delayed tumor growth	(52)
	Lymphoma	Tet-off [§]	Tumor regression	unpublished
		PNA, nanoparticle	Delayed tumor growth	unpublished
miR-380-5p	Neuroblastoma	LNA	Delayed tumor growth	(44)

Abbreviations: antagomiR, cholesterol-conjugated RNA with 2'-O-Me and phosphorothioate modifications; PNA, peptide nucleic acid; LNA, locked nucleic acid; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; DLBCL, diffuse large B cell lymphoma

^{*} No therapeutic effect observed on the primary tumor

[†] Chemistry and transfection method not described

[‡] Delivered in combination with transduction of apoptosis-inducing S-TRAIL

[§] miR-21 knockout mice crossed with transgenic mice harboring activated Kras

^{**} Conditional miRNA overexpression turned off by doxycycline

^{††} Tumorigenesis requires coordination with activated Kras

^{‡‡} HCV infection promotes HCC, study conducted using non-human primates

^{§§} Anti-miR inserted into PLKO lentiviral expression vector

^{***} Anti-TNF α treatment to inhibit induction of miR-155