Role of MicroRNAs-221/222 in Digestive Systems

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Abstract: MiR-221 and miR-222 (miR-221/222) are well-studied oncogenic microRNAs that are frequently upregulated in several types of human tumors, such as esophageal adenocarcinoma, gastric adenocarcinoma, colorectal adenocarcinoma, hepatocellular carcinoma, and pancreatic ductal adenocarcinoma. In these cancers, silencing miR-221/222 could represent a novel anti-tumor approach to inhibit tumor growth and metastasis. On the other hand, miR-221/222 also play onco-suppressive roles in cholangiocarcinoma and gastrointestinal stromal tumors (GISTs). Here we will review the roles of miR-221/222 in digestive systems and their possibility as prognostic and therapeutic tools.

Keywords: microRNA; colorectal cancer; hepatocellular carcinoma; pancreatic cancer

1. Introduction

MicroRNAs (miRs) are ~22 nucleotide noncoding RNAs that can downregulate various gene products by translational repression when partially complementary sequences are present in the 3′ untranslated regions (3′ UTR) of the target mRNAs or by directing mRNA degradation. Increasing evidence has demonstrated that miRs are involved in cancer initiation, progression, and metastasis, and may serve as diagnostic and prognostic biomarkers for cancers. Among the many miRNAs already identified as regulators of neoplastic transformation, invasion, and metastasis, miR-221 and miR-222...
(miR-221/222) have emerged as key miRNAs deregulated in many cancers, such as gastrointestinal cancers, breast cancer, prostate cancer, thyroid cancer, and glioma [1–5]. MiR-221 and miR-222 are encoded in tandem from a gene cluster located on chromosome Xp11.3. Several reports indicated that miR-221/222 could be used as a therapeutic tool to decrease cell proliferation or modulate sensitivity to anti-cancer agents [6–8]. Here we review the current knowledge about the role of miR-221/222 in digestive systems, including hepatobiliary and pancreatic cancers.

2. Direct Targets of miR-221/222

The identification of target mRNAs is a key step for assessing the role of aberrantly expressed microRNAs in human cancer. To date, various direct targets of miR-221/222 have been reported, even in the digestive system, as shown in Table 1. Among them, regulation of p27Kip1 by miR-221/222 is well studied. Downregulation of p27Kip1 is required for cell cycle entry after growth factor stimulation. MiR-221/222 are underactive towards p27Kip1-3′ UTRs in quiescent cells, as a result of target site hindrance. Pumilio-1 (PUM1) is a ubiquitously expressed RNA-binding protein (RBP) that interacts with p27Kip1-3′ UTR. In response to growth factor stimulation, PUM1 is upregulated and phosphorylated for optimal induction of its RNA-binding activity towards the p27Kip1-3′ UTR [9]. PUM1 binding induces a local change in RNA structure that favors association with miR-221/222, efficient suppression of p27Kip1 expression, and rapid entry to the cell cycle.

Table 1. Direct targets of miR-221/222.

| Target  | Cancer Type                        | Reference                                                                 |
|---------|------------------------------------|---------------------------------------------------------------------------|
| p27Kip1 | Esophageal adenocarcinoma          | Matsuzaki et al. (2013)                                                   |
|         | Hepatocellular carcinoma           | Pineau et al. (2010), Fu et al. (2011), Callegari et al. (2012)            |
|         | Pancreatic ductal adenocarcinoma   | Park et al. (2009), Sarkar et al. (2013), Tanaka et al. (2015)             |
| p57Kip2 | Colorectal ductal adenocarcinoma   | Sun et al. (2011)                                                         |
|         | Pancreatic ductal adenocarcinoma   | Sarkar et al. (2013)                                                      |
| PTEN    | Gastric adenocarcinoma             | Chun-Zhi et al. (2010)                                                    |
|         | Hepatocellular carcinoma           | Fornari et al. (2008), Callegari et al. (2012), Garofalo et al. (2009)    |
|         | Colorectal adenocarcinoma          | Tsunoda et al. (2011), Xue et al. (2013)                                  |
| RelA    | Colorectal adenocarcinoma          | Liu et al. (2014)                                                         |
| PDLIM2  | Colorectal adenocarcinoma          | Liu et al. (2014)                                                         |
| RECK    | Colorectal adenocarcinoma          | Qin et al. (2014)                                                        |
| BMF     | Hepatocellular carcinoma           | Gramantieri et al. (2009), Callegari et al. (2012), He et al. (2014)       |
| BBC3    | Hepatocellular carcinoma           | He et al. (2014)                                                          |
| ANGPTL2 | Hepatocellular carcinoma           | He et al. (2014)                                                          |
| HDAC6   | Hepatocellular carcinoma           | Bae et al. (2015)                                                         |
| ERα     | Hepatocellular carcinoma           | Chen et al. (2015)                                                        |
| SOCS1   | Hepatocellular carcinoma           | Xu et al. (2014)                                                          |
| SOCS3   | Hepatocellular carcinoma           | Xu et al. (2014)                                                          |
| MDM2    | Hepatocellular carcinoma           | Formari et al. (2014)                                                    |
| DDIT4   | Hepatocellular carcinoma           | Pineau et al. (2010)                                                     |
Table 1. Cont.

| Target | Cancer Type                        | Reference                  |
|--------|-----------------------------------|----------------------------|
| TIMP3  | Hepatocellular carcinoma          | Garofalo et al. (2009)     |
| TIMP2  | Pancreatic ductal adenocarcinoma  | Xu et al. (2015)           |
| PIK3R1 | Colangiocarcinoma                 | Okamoto et al. (2013)      |
| PUMA   | Pancreatic ductal adenocarcinoma  | Sarkar et al. (2013)       |
| TRPS1  | Pancreatic ductal adenocarcinoma  | Su et al. (2013)           |
| KIT    | Gastrointestinal stromal tumor    | Koelz et al. (2011), Gits et al. (2013), Ihle et al. (2015) |

3. Esophageal Cancer

Duodenogastro-esophageal bile reflux contributes to development of esophageal adenocarcinoma. We recently reported that expression levels of miR-221/222 increased, along with the activity of nuclear bile acid receptor/farnesoid X receptor (FXR), when cultured esophageal epithelial cells were exposed to bile acids [10]. Furthermore, miR-221/222 expression was higher in esophageal adenocarcinoma than in the surrounding Barrett’s esophagus, a precursor lesion of esophageal adenocarcinoma. p27Kip1 is known to inhibit the proteasomal protein degradation of the transcription factor CDX2. We also confirmed that the levels of p27Kip1 and CDX2 were lower in areas of esophageal adenocarcinoma than in those of Barrett’s esophagus. Incubation of cells with bile acids increased degradation of CDX2; this process was reduced when cells were also incubated with proteasome inhibitors. Overexpression of miR-221/222 reduced levels of p27Kip1 and CDX2, and knockdown of miR-221/222 increased levels of these proteins in cultured cells. In addition, inhibitors of miR-221/222 reduced growth of xenograft tumors in immunodeficient mice.

4. Gastric Cancer

Liu et al. reported that miR-221 was upregulated in 88% of gastric cancer tissue samples compared with their paired adjacent non-tumor tissue samples [11]. High expression of miR-221 showed a significant correlation with advanced tumor-node-metastasis stage, local invasion, and lymphatic metastasis. MiR-221 overexpression was an unfavorable prognostic factor for overall survival in patients with gastric cancer. In gastric cancer cells, upregulation of miR-221/222 induced the malignant phenotype, whereas knockdown of miR-221/222 reversed this phenotype via induction of PTEN, a direct target of miR-221/222 [12]. In addition, knockdown of miR-221/222 inhibited cell growth and invasion and increased the radiosensitivity.

5. Colorectal Cancer

MiR-221 was upregulated in 90% of colorectal cancer (CRC) tissue samples compared to that in the adjacent non-tumorous tissue, and the expression level was positively correlated to an advanced TNM stage and local invasion [13–18]. A survival analysis indicated that high expression of miR-221 was closely associated with a shorter survival time [14,19]. In CRC cells, miR-221 overexpression enhances, whereas miR-221 depletion reduces CRC cell proliferation, migration, invasion, and colony formation [16,17]. In mice with colitis, injection of lentiviruses expressing miR-221/222 sponges led to
formation of fewer tumors than injection of control lentiviruses [16]. Protein expressions of p57Kip2 and RECK, direct targets of miR-221, were decreased in the CRC tissues, and promoted CRC occurrence and progress [15,17].

Liu et al. reported that mimics of miR-221/222 activated NF-κB and STAT3 in CRC cells [16]. MiR-221/222 also reduced the ubiquitination and degradation of the RelA and STAT3 proteins by binding to the 3' untranslated region of PDLIM2 mRNA (PDLIM2 is a nuclear ubiquitin E3 ligase for RelA and STAT3). In human CRC tissues, levels of miR-221/222 positively correlated with levels of RelA and STAT3 mRNAs. Levels of PDLIM2 mRNA were lower than non-tumor tissues.

Xue et al. investigated the regulative effect of miR-221 on CRC cell radiosensitivity [20]. X-ray radiation had an effect on the expression of miR-221 in CRC cells in a dose-dependent manner. The protein levels of PTEN, a direct target of miR-221, reduced gradually during exposure to X-rays. Inhibition of miR-221 upregulated expression of PTEN protein and enhanced the radiosensitivity. Moreover, the inhibitory effect was dramatically abolished by pretreatment with anti-PTEN-siRNA, suggesting that the enhancement of radiosensitivity was mediated by PTEN.

Tsunoda et al. reported that the increased expression of miR-221/222 was observed in 3D culture as compared with 2D culture [18]. They showed that miR-221/222 was regulated by oncogenic KRAS, which plays several key roles in 3D culture. The protein expression level of PTEN was reduced under the control of KRAS in a 3D-specific manner.

The plasma concentration of miR-221 is a potential biomarker for differentiating CRC patients from controls [21]. Kaplan–Meier curve assessment shows that the elevated plasma miR-221 level is a significant prognostic factor for poor overall survival in CRC patients. The immunohistochemistry analysis demonstrates a significant correlation between plasma miR-221 level and p53 expression.

Stool-based miR-221 can also be used as a non-invasive biomarker for the detection of CRC [13]. In stool samples, miR-221 showed a significant increasing trend from normal controls to late stages of CRC. The AUC of stool miR-221 was 0.73 for CRC patients as compared with normal controls. No significant differences in stool miR-221 levels were found between patients with proximal and distal CRCs. The use of antibiotics did not influence stool miR-221 levels.

6. Hepatocellular Carcinoma

MiR-221/222 is a critical modulator in the hepatocellular carcinoma (HCC) signaling pathway [22]. MiR-221/222 was upregulated in the human liver in a fibrosis progression-dependent manner with upregulation of α1 (I) collagen (COL1A1) and α-smooth muscle actin (αSMA) [23–25]. Upregulation of miR-221 and downregulation of p27Kip1 and p57Kip2 were associated with tumor stages, local recurrence, metastasis, and poor prognosis [24–29]. In a mouse model of liver cancer, miR-221 overexpression stimulated growth of tumorigenic murine hepatic progenitor cells [30,31]. Inhibition of miR-221 decreased liver cancer cell proliferation, clonogenicity, migration, and invasion and also induced G1 arrest and apoptosis in vitro and in vivo [22,27,32].

In HCC cells or hepatocyte, various functions of miR-221/222 have been investigated (Figure 1). In addition to p27Kip1 and p57Kip2, several direct targets of miR-221/222 were identified, such as estrogen receptor-alpha (ERα) and a proapoptotic BH3-only protein (BMF) [33,34]. DNA damage-inducible transcript 4 (DDIT4), a modulator of mTOR pathway, was also a direct target of
miR-221 [30]. Garofalo et al. showed that miR-221/222, by targeting PTEN and TIMP3 tumor suppressors, induce TRAIL resistance and enhance cellular migration through the activation of the AKT pathway and metallopeptidases. Xu et al. reported that miR-221 was upregulated by HCV infection [35]. In addition, an miR-221 mimic could accentuate the anti-HCV effect of IFN-α in an HCV model, through the inhibition of two members of the suppressor of cytokine signaling (SOCS) family, SOCS1 and SOCS3.

In HCC cells, regulation systems of miR-221/222 have also been investigated (Figure 1). JNK/c-Jun activation and NF-κB nuclear translocation were reported to be essential for the transcription of miR-221/222 [23,36,37]. Hepatitis B virus X protein (HBx) leads to the promotion of cell proliferation and cell growth viability with overexpression of miR-221 [33]. HCV infection could also upregulate the expression of miR-221 in an NF-κB dependent manner [35,38]. Staphylococcal nuclease domain-containing 1 (SND1) is a multifunctional protein that is overexpressed in multiple cancers, including hepatocellular carcinoma (HCC). Santheladur et al. reported that SND1-induced activation of NF-κB resulted in induction of miR-221 and subsequent induction of angiogenic factors Angiogenin and CXCL16 [39].

Figure 1. A schematic of the regulatory mechanisms of miR-221/222 in hepatocarcinogenesis.

Bae et al. showed that the direct suppression of HDAC6 (histone deacetylase 6) by miR-221 was induced by JNK/c-Jun signaling in liver cancer cells but not in normal hepatic cells [36]. In addition, NF-κB could be activated by miR-221, since HDAC6 suppressed the translocation of NF-κB.

Fornari et al. reported that MDM2 (E3 ubiquitin-protein ligase homolog), a known p53 (TP53) modulator, is identified as a direct target of miR-221 [40]. MiR-221 can activate the p53/mdm2 axis by inhibiting MDM2 and, in turn, p53 activation contributes to miR-221 enhanced expression. Giovannini et al. reported that Notch3 silencing in HCC resulted in p53 upregulation [41]. They found
that Notch3 regulated p53 at post-transcriptional level controlling both Cyclin G1 expression and the feed-forward circuit involving p53, miR-221, and MDM2.

7. Pancreatic Cancer

Expression of miR-221/222 is upregulated in pancreatic cancer as compared with normal pancreatic duct epithelial cells or normal pancreas tissues [42–44]. Pancreatic cancer patients with high miR-221 expression had a relatively shorter survival compared to those with lower expression [42]. Antisense to miR-221 suppressed the proliferative capacity, increased the amount of apoptosis, and sensitized the effects of gemcitabine in pancreatic cancer cells with concomitant up-regulation of PTEN, p27Kip1, p57Kip2, and PUMA, which are the tumor suppressors and the predicted targets of miR-221 [42,45,46].

Tanaka et al. reported that metformin suppressed the expression of miR-221 in human pancreatic cancer cells, leading to G1-phase arrest via the upregulation of p27Kip1 [47]. In addition, Sarker et al. reported that the treatment of pancreatic cancer cells with isoflavone mixture (G2535), formulated 3,3′-diindolylmethane (BR-DIM), or synthetic curcumin analogue (CDF) could downregulate the expression of miR-221 and consequently upregulate the expression of PTEN, p27Kip1, p57Kip2, and PUMA, leading to the inhibition of proliferation and migration of pancreatic cancer cells [42]. Therefore, these agents combined with conventional chemotherapeutics could be useful in designing novel targeted therapeutic strategy for the treatment of pancreatic cancer.

Matrix metalloproteinases (MMPs) are closely related to cell migration and invasion. Among the MMPs, MMP-2 and MMP-9 have been implicated in human cancer invasion. Xu et al. reported that the tissue inhibitor of metalloproteinase (TIMP)-2 was directly regulated by miR-221/222 [43]. They also showed that miR-221/222 mimic directly inhibited TIMP-2 expression, leading to the upregulation of MMP-2 and MMP-9.

The platelet-derived growth factor (PDGF) signaling pathway has been found to play important roles in the development and progression of human cancers by regulating the processes of cell proliferation, apoptosis, migration, invasion, metastasis, and the acquisition of the epithelial-mesenchymal transition (EMT) phenotype. Su et al. reported that miR-221 expression was activated by PDGF signaling [48]. After the inhibition of miR-221, PDGF did not alter the levels of cell migration, proliferation, and acquisition of the EMT phenotype. These results showed that miR-221 is essential for the PDGF-mediated EMT phenotype, migration, and growth of pancreatic cancer cells. Downregulation of TRPS1 by miR-221 is critical for PDGF-mediated acquisition of the EMT phenotype.

Plasma miR-221 concentration could be a useful biomarker for cancer detection, monitoring tumor dynamics, and predicting malignant outcomes in pancreatic cancer patients [44]. Plasma miR-221 levels were higher in pancreatic cancer patients than in benign pancreatic tumors and controls, and were correlated with distant metastasis. In addition, plasma miR-221 levels were reduced in postoperative samples.

Pancreatic cysts are a group of lesions with heterogeneous malignant potential. MiR-221 concentration in the endoscopically acquired pancreatic cyst fluid samples could be useful for the diagnosis of pancreatic cysts. MiR-221 was expressed at higher levels in malignant cysts compared with benign or premalignant cysts [49].
8. Cholangiocarcinoma

In contrast to the other epithelial cancers, miR-221/222 was downregulated in intrahepatic cholangiocarcinoma tissues, suggesting that miR-221/222 would play onco-suppressive roles [25]. Okamoto et al. reported a relationship between miR-221 expression and the sensitivity of cholangiocarcinoma (CCA) cells to gemcitabine [50]. Microarray analysis was used to determine the miRNA expression profiles of two CCA cell lines, HuCCT1 and HuH28. HuCCT1 cells were more sensitive to gemcitabine than were HuH28 cells, and 18 miRNAs were differentially expressed between HuH28 and HuCCT1. To determine the effect of candidate miRNAs on gemcitabine sensitivity, expression of each candidate miRNA was modified via either transfection of a miRNA mimic or transfection of an anti-oligonucleotide. Among these 18 miRNAs, ectopic overexpression of each of three downregulated miRNAs in HuH28 (miR-29b, miR-205, and miR-221) restored gemcitabine sensitivity to HuH28. Selective siRNA-mediated downregulation of either of two software-predicted targets, PIK3R1 (target of miR-29b and miR-221) or MMP-2 (target of miR-29b), also conferred gemcitabine sensitivity to HuH28.

9. Gastrointestinal Stromal Tumor (GIST)

Gastrointestinal stromal tumors (GISTs) are characterized by high expression of the KIT receptor tyrosine kinase protein, resulting from oncogenic mutations in the extracellular, juxtamembrane, or kinase domains. KIT is known to be directly regulated by miR-221/222, suggesting that miR-221/222 would also play onco-suppressive roles in GISTs [51]. In fact, expression of miR-221/222 is reduced in GISTs compared to control tissue and other sarcomas [51–53]. Overexpression of miR-221/222 in GIST cells inhibited cell proliferation, affected cell cycle progression, and induced apoptosis [51,52]. Ihle et al. analyzed expression of miR-221/222 in six KIT exon 9, three KIT exon 11 mutated, and nine wild-type GISTs [52]. MiRNA expression was lower for the wild-type compared to mutated GISTs. Transient transfection of miR-221/222 reduced viability and induced apoptosis by inhibition of KIT expression and its phosphorylation and activation of caspases 3 and 7 in GIST cells. p-AKT, AKT, and BCL2 expression were also reduced after miR-221/222 transfection.

10. Conclusions and Prospects

The discovery of the important role of miRNAs in cancer has opened up a new era of cancer investigations that take into account new and emerging knowledge regarding the RNA signaling systems. The unraveling of miR-221/222 signaling pathways and networks will be key to understanding the role that deregulated miRNA functioning can play in oncogenic or onco-suppressive processes and may be important for defining novel therapeutic molecules. Recently miRNAs contained in exosomes have been shown to be released and to act as a signal transducer. However, the function of secretory miR-221/222 has never been reported. Previous reports showed that miR-221/222 play various roles not only in cancer but also in vascular smooth muscle cells, vascular endothelial cells, and adipose tissue [54,55]. These suggest that interactions between cancers and blood vessels or adipose tissue would be mediated by secretory miR-221/222. Revealing the inter-organic functions of miRNAs will also help us to better understand cancer biology.
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Author Contributions

Juntaro Matsuzaki wrote and Hidekazu Suzuki supervised and revised the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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