Published in final edited form as:

Curr Mol Med. 2012 January ; 12(1): 27–33.

miR221/222 in Cancer: Their Role in Tumor Progression and Response to Therapy

M. Garofalo¹, C. Quintavalle², G. Romano³, C.M. Croce¹, and G. Condorelli^{*2}

¹Department of Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, Ohio State University, Columbus, OH, USA

²Department of Cellular and Molecular Biology and Pathology, "ederico II" University of Naples, Biotechnological Science Faculty, and IEOS, CNR Naples, Italy

³Fondazione IRCCS-SDN, Naples, Italy

Abstract

MiRNAs are small non-coding RNAs of ~24 nt that can block mRNA translation and/or negatively regulate its stability. There is a large body of evidence that dysregulation of miRNAs is a hallmark of cancer. miRNAs are often aberrantly expressed and their function is linked to the regulation of oncogenes and/or tumor suppressor genes involved in cell signaling pathway. MiR-221 and miR-222 are two highly homologous microRNAs, whose upregulation has been recently described in several types of human tumors. MiR-221/222 have been considered to act as oncogenes or tumor suppressors, depending on tumor system. Silencing oncomiRs or gene therapy approaches, based on re-expression of miRNAs that are down-regulated in cancer cells, could represent a novel anti-tumor approach for integrated cancer therapy. Here we will review the role of miR-221/222 in cancer progression and their use as prognostic and therapeutic tools in cancer.

Keywords

microRNA; cancer; cancer therapy

INTRODUCTION

MicroRNAs (miRNAs) are small ~22nt single stranded RNAs that negatively regulate protein expression by binding to partially complementary sequences in the 3' untranslated region (3' UTRs) of target gene messenger RNAs (mRNA). MiRNAs are generated by a mechanism that typically involves the transcription of a long precursor (pri-miRNA), which is then processed to produce a second precursor (pre-miRNA) of 60–100 nucleotides in length by the nuclear protein Drosha (Fig. 1). The pre-miRNA is transported to the cytoplasm and a second processing step is carried out by an endoribonuclease of the RNase III family, Dicer, resulting in the final miRNA products [1]. In the cytoplasm, miRNA duplexes are unwound and the mature strand is incorporated into RNA-induced silencing complex (RISC). Following binding to partially complementary sites, usually in the 3' UTRs of mRNAs, miRNAs cause an inhibition of translation and, in some cases, the degradation of the target mRNA (Fig. 1) [2]. There are hundreds of miRNAs encoded in the

human genome and thousands of target mRNAs, and this explains the important regulatory roles of miRNAs in cell developmental, differentiation, proliferation and apoptotic pathways. Therefore, deregulated miRNAs are involved in the pathogenesis of many human diseases, including cancer. Indeed, both loss and gain of miRNA function contribute to cancer development through a range of different mechanisms. Since miRNAs regulate cancer cell differentiation, proliferation, survival, and metastasis, the regulation of their expression levels may provide a powerful therapeutic strategy toward cancer initiation and progression. MicroRNAs are aberrantly expressed in various cancer types including leukemia [3, 4], lymphoma [5] breast cancer [6, 7], colorectal cancer [8], lung cancer [9, 10], hepatocellular carcinoma [11, 12], and oral cancer [13–15]. Deregulation (*e.g.*, overexpression or loss of expression) of these “cancerous” microRNAs contributes to tumor initiation and progression by favoring uncontrolled proliferation and survival, promoting invasive behavior, and by regulating apoptosis [16, 17]. An increasing number of studies have then demonstrated that microRNAs can function as potential oncogenes (*oncomiRs*) or oncosuppressor genes (*oncosuppressor-miRs*), depending on the cellular context and on the target genes. In this regard, miR-221/222 are overexpressed in the majority of epithelial tumors [18] but they can play a tumor-suppressive role in erythroleukemic cells by inhibiting erythropoiesis through the downregulation of c-Kit receptor. Here we review the current knowledge about the role of miR-221/222 in tumor development and their potential as diagnostic, prognostic and therapeutic tools.

MiR-221/222 Role in Tumor Progression

miR-221/222 are encoded in tandem on the X chromosome in human, mouse and rat and are highly conserved in vertebrates. Moreover, they have the same seed sequence. Many studies to date have been reported on the role of miR-221/222 in cancer development either as oncomiR or as oncosuppressor-miRs (see Table 1 and 2). Overexpression of miR-221/222 has been observed in a number of advanced malignancies indicating that miR-221/222 could be potential therapeutic targets for epithelial cancer [19]. Galardi *et al.*, identified the cell cycle regulator, p27^{Kip1}, as a target of miR-221/miR-222 [20]. They showed that in pancreatic cells, p27^{Kip1} and miR-221/222 expression levels inversely correlated and demonstrated that miR221/222 overexpression had important consequences on the proliferation rate and the cell cycle phase distribution. These results were then confirmed in glioblastomas [21], in thyroid papillary carcinomas [22], in breast cancer [23], hepatocellular carcinoma [24], and lung cancer [25]. Afterwards many other tumor suppressor genes have been identified as miR-221/222 targets. Fornari *et al.*, reported that CDKN1C/p57, is also a direct target of miR-221 in the liver, suggesting that miR-221 has an oncogenic function in hepatocarcinogenesis. Indeed, transfection of miR-221 into Hep3B cells caused a 1.8-fold decrease of CDKN1C/p57; conversely, SNU449 cells transfected with anti-miR-221 exhibited a 1.3-fold increase in CDKN1C/p57 protein levels when compared with negative control miRNA inhibitors [26]. Zhang *et al.*, reported that miR-221/222 directly regulate apoptosis by targeting PUMA in glioblastoma; moreover, they found an inverse relationship, *in vivo*, between PUMA and miR-221/222 expression in glioma tissues [19]. More recently, we have demonstrated that miR-221/222 are overexpressed in high grade glioma patients and regulate the expression of the tyrosine phosphatase PTP μ [27]. Since PTP μ negatively regulates glioma cells migration, miR-221/222 overexpression has important consequences in glioma tumorigenesis. Moreover, our recent data suggest that miR-221/222 are also able to regulate temozolomide response (Quintavalle, personal communication), by modulating the expression of the DNA repair enzyme, MGMT. By decreasing the expression of MGMT, miR-221/222 render glioma cells more responsive to temozolomide. Therefore, if on one hand miR-221/222 are found overexpressed in highly invasive tumor, on the other hand, patients with higher miR-221/222 levels, could respond better to the therapy. Zhao *et al.*, found frequent up-

regulation of miR-221/222 in ER- α negative breast cancer cell lines and in primary tumors and demonstrated that those miRs inhibit ER- α translation by direct interaction with the 3'-UTR of ER- α , providing a molecular mechanism of ER- α posttranscriptional regulation in breast cancer [28]. Di Leva *et al.*, demonstrated a feedback loop within miR-221/222 and ER- α . They showed that miR-221/222 inhibit the expression of ER- α and FOXO3A transcription factors, and that ER- α is able to repress miR-222/221 transcriptional activation. Repression of FOXO3 by miR-221/222, in turn, blocks transcriptional activation of p27 and Bim (Fig. 2). These findings provide evidence that a single miRNA, through its ability to modulate different genes involved in the same network, may act as a strong inhibitor of the entire cellular pathway, suggesting a possible greater therapeutic potential for miRNAs than for a single gene [29]. Terasawa *et al.*, found that nerve growth factor (NGF)-driven activation of the extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathway in prostate cancer cells, induced expression of miR-221/222. Using a target prediction program, they identified the pro-apoptotic protein Bim, as a potential miR-221/222 target [30]. MicroRNA function depends on the cellular context. Indeed, miR-222 inhibits oral tongue squamous cell carcinoma (OTSCC) cell invasion. Ectopic transfection of miR-222 reduced the expression of matrix metalloproteinase 1 (MMP1) and manganese superoxide dismutase (SOD2), both implicated in cell invasion and metastasis, in OTSCC cell lines (Fig. 3). These results indicate that miR-222 plays an important role in OTSCC invasion, and may thus serve as a novel therapeutic target for high risk metastatic OTSCC patients [31]. Another study from Felli *et al.*, indicated that miR-221/222 inhibit normal erythropoiesis and erythroleukemic cell growth at least in part *via* Kit receptor down-modulation. Indeed, in erythropoietic culture of cord blood CD34+ progenitor cells, miR-221/222 levels are markedly down-modulated. In erythropoietic culture undergoing exponential cell growth, miR down-modulation is inversely related to increasing Kit protein expression. Treatment of CD34+ progenitors with miR-221/222 is able to induce impaired proliferation and increased differentiation of E cells [32]. These last two studies evidence a tumor suppressive role for miR-221/222 in OTSCC and erythrocytes, indicating again that microRNA function is exclusively dependent on the cellular context and tumor type (Fig. 3).

MiR-221/222: Their Role to Response to Therapy in Cancer

Recent studies suggest that chemoresistance may be linked to dysregulation of microRNA function. Furthermore, mounting evidence indicates the existence of similarities between drug-resistant and metastatic cancer cells in terms of resistance to apoptosis and enhanced invasiveness. miR221/222 overexpression is linked to altered response to cancer therapy, either to hormone-based or to conventional chemotherapy. Studies indicate that miR-221/222 have a prominent role in the acquisition of anti-estrogen resistance. Estrogen receptor- α (ER α), plays a pivotal role in the development and progression of breast cancer [33]. Tamoxifen, a selective estrogen receptor modulator [34], is considered to be the first line endocrine therapy that greatly improves relapse-free and overall survival of ER-positive breast cancer at all stages [35]. However, acquired resistance to tamoxifen typically develops after prolonged treatment in a majority of initially responsive breast cancers [36]. Moreover, nearly 50% of the advanced ER-positive breast cancer patients do not respond to tamoxifen in the first line setting. From the analysis of miR-221/222-regulated gene expression profiles emerged that resistance to tamoxifen is associated with activation of several growth factor signaling pathways, including human epidermal growth factor receptor 2 (HER2) and insulin-like growth factor I receptor (IGF-IR), that may 'cross-talk' with the ER α signaling pathway. This results in an activation of the mitogen-activated protein kinases (MAPKs) and PI3K/AKT involved in cell survival and proliferation [35–37]. Knockdown of miR-221 and/or miR-222 sensitized MDA-MB-468 cells to tamoxifen-induced cell growth arrest and apoptosis. These findings indicate that miR-221/222 play a significant role in the regulation of ER α expression and could be potential targets for

restoring ER α expression and responding to anti-estrogen therapy in a subset of breast cancers [28]. Other studies demonstrate that miR-221/222 induced fulvestrant resistance in MCF-7 sensitive cells through the deregulation of multiple oncogenic signaling pathways such as the activation of β -catenin and repression of TGF- β -signaling-induced growth inhibition [38] (Fig. 2). Therefore, targeting these two oncomiRs may be a potential therapeutic strategy for preventing the development of fulvestrant resistance or resensitizing breast tumors to this potent estrogen antagonist. Felicetti *et al.*, reported that the promyelocytic leukemia zinc finger (PLZF) transcription factor is a repressor of miR-221/222 by direct binding to their putative regulatory region. Specifically, they demonstrated that PLZF silencing in melanomas unblocks miR-221/222, which, in turn, enhanced proliferation and differentiation blockade of the melanoma cells through the down-modulation of p27^{Kip1} and c-KIT receptor. *In vitro* and *in vivo* functional studies, including the use of antisense "antagomiR" oligonucleotides, confirmed the key role of miR-221/222 in regulating the progression of human melanoma [39]. Pu *et al.*, recently reported that plasma levels of miR-221 are a potential biomarker for differentiating colorectal cancer (CRC) patients from controls. Indeed, the elevated plasma miR-221 level is a significant prognostic factor for overall survival in CRC patients and can be used as a potential molecular marker for diagnosis and prognosis of CRC [40]. Chen *et al.*, analyzed different thyroid tumors and, interestingly, they found miR-221/222 overexpressed mostly in papillary thyroid carcinomas [41]. Gottardo *et al.*, identified miR-221 upregulation in bladder cancer compared to bladder normal mucosa, implying the role of miR-221 as prognostic tool also for bladder cancer [42]. Our group showed that in TRAIL-resistant NSCLC cells, levels of miR-221/222 are increased. Transfection with anti-miR-221 and -222 rendered CALU-1-resistant cells sensitive to TRAIL; conversely, H460-sensitive cells treated with miR-221/222 become TRAIL-resistant. We reported that miR-221/222 interfere with TRAIL signaling mainly through p27^{Kip1} (Fig. 2). Thus, high expression levels of miR-221/222 are needed to maintain the TRAIL-resistant phenotype, making these miRs as promising diagnostic tool for TRAIL resistance in NSCLC [25]. Moreover, an interesting regulation loop of miR-221/222 and TRAIL sensitivity has been recently described by Acunzo *et al.*, [43]. In fact, it has been reported that miR-130 overexpression is able to downregulate miR-221/222 expression through MET down modulation. This finding highlighted how miR-130a, by targeting MET, is able to reduce miR-221/222 expression and, accordingly, TRAIL resistance in NSCLC cells and strongly confirms the involvement of miR-221/222 in the regulation of TRAIL sensitivity.

Indeed, tumor stratification, on the basis of miR-221/222 expression levels, could be used as prognostic tool to predict TRAIL-sensitivity or TRAIL-resistance in the treatment of NSCLCs [25].

miR-221/222 as Therapeutic Tool

Several reports indicate that miR-221/222 could be used as therapeutic tool to modulate resistance or sensitivity to anti-cancer agents. Our group recently found that MET, through Jun transcriptional activation, upregulates miR-221/222 expression, which, in turn, by targeting PTEN and TIMP3, confers resistance to TRAIL-induced cell death and enhances tumorigenicity of lung and liver cancer cells [44]. PTEN is a lipid phosphatase that directly antagonizes the activity of phosphatidylinositol 3-OH kinase (PI3K) [45]. Its inactivation results in constitutive activation of the PI3K/AKT pathway and in subsequent increase in protein synthesis, cell cycle progression, migration, and most importantly survival [46]. TIMP3 belongs to the family of the tissue inhibitors of metalloproteinases (TIMPs), of which there are four family members (TIMP1–4) [47]. They inhibit the activity of metalloproteinases (MMPs) by binding with a 1:1 stoichiometry to the active site [48], but other studies showed that TIMP3 promotes apoptotic cell death [49, 50].

Another study from Chun-Zi *et al.*, demonstrated that miR-221 and miR-222 regulate radiosensitivity, cell growth and invasion of gastric cells, *via* direct modulation of PTEN expression [51]. These results suggest that inhibition of miR-221 and miR-222 might form a novel therapeutic strategy finalized not only to sensitize tumor cells to drug-inducing apoptosis but also to inhibit their survival, proliferation, and invasive capabilities.

Recently, Pineau *et al.*, [52] identified DNA damage-inducible transcript 4 (DDIT4), a modulator of the mTOR pathway, as target of miR-221. They introduced into liver cancer cells, LNA-modified antimiR-221 and antimiR-222. Treatment by antagomiRs, but not scrambled oligonucleotides, reduced the growth of liver cancer cells that overexpressed miR-221/222. Thus the use of synthetic inhibitors of miR-221 may prove to be a promising approach to liver cancer treatment [52]. Galardi *et al.* showed that miR-221/222 knockdown through antisense LNA oligonucleotides increase p27^{Kip1} in human prostate cancer (PC3) cells and strongly reduce their clonogenicity in *in vitro*, whereas xenograft model in SCID mice demonstrated that the ectopic overexpression of miR-221 may confer a high growth advantage. Consistently, treatment of established subcutaneous tumors with antago-miR 222/221 reduces tumor growth by increasing intratumoral p27 amounts [20]. Interestingly, Wang *et al.* designed adenovirally-expressed shRNAs that functionally co-repressed the expression of miR-221/222 in glioblastoma cells, upregulating p27 expression levels, inducing cell cycle arrest in G1 phase and apoptosis [53]. Finally, Pogribny *et al.*, studied the role of miRNA alterations in the acquisition of cisplatin-resistant phenotype in MCF-7 human breast adenocarcinoma cells. They identified a total of 103 miRNAs (46 upregulated and 57 downregulated) in MCF-7 cells resistant to cisplatin. Among the most upregulated miRs they found miR-221/222, involved in the control of cell signaling and cell survival [54]. Our recent data demonstrate that miR-221 and -222 are up-regulated in breast cancer stem cells obtained from patients or from cultured MCF7 cells grown in suspension (Condorelli personal communication). Their role in cancer stem cells behavior is under investigation.

CONCLUSION

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 within eukaryotic cells such as mammalian cells. Among the many miRNAs already identified as regulators of neoplastic transformation, invasion and metastasis, miR-221/222 have emerged as key miRNAs deregulated in many cancers. Even though miR-221/222 are among the most intensively studied miRNAs, our understanding of their signaling pathways is currently in its early stages. The unraveling of such RNA signaling pathways and networks will be a key to understand the role that deregulated miRNA functioning can play in oncogenic processes and may be important for defining novel therapeutic molecules. Examples of such therapeutics include silencing oncomiRs or gene therapy approaches based on reexpression of miRNAs that are down-regulated in cancer cells. Still many studies are necessary to translate these findings from *in vitro* to *in vivo* and to develop successful *in vivo* delivery systems based on organ specificity, less or no toxicity and no off-target effects.

Acknowledgments

This work was partially supported by funds from Associazione Italiana Ricerca sul Cancro, AIRC to GC (grant n.ro 10620), MERIT (RBNE08E8CZ_002) to GC, by the Sidney Kimmel Cancer Research Foundation (MG) and by Programma Operativo Nazionale (PON 01_01602) to GC C.Q. is recipient of a FIRC (Federazione Italiana Ricerca sul Cancro) fellowship.

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Fig. (1). microRNA biogenesis

The production of microRNAs (miRNAs) from pri-miRNA is a complex and coordinated process operated by different groups of enzymes and associated proteins in the nucleus or cytoplasm. The pri-miRNA, located in the nucleus, is converted in pre-miRNA through the cleavage activity of the Drosha enzyme. The produced pre-miRNA is exported to the cytoplasm by the Exportin 5. Upon its arrival into the cytoplasm, the pre-miRNA is processed in ~18–22-nucleotide miRNA duplexes by the cytoplasmic RNase-III Dicer. Usually, one strand of this duplex is degraded, whereas the other strand accumulates as a mature miRNA. From the miRNA-miRNA duplex, only the miRNA enters preferentially in the protein effector complex, formed by the RNA-induced silencing complex (RISC).

Perfect or nearly perfect complementarities between miRNA and its target 3' UTR induce RISC to cleave the target mRNA, whereas imperfect base matching induces mainly translational silencing of the target.

Fig. (2). MiR-221/222 as oncomiRs

MiR-221/222 act as oncomiRs by targeting important tumor suppressor genes such as PTEN, TIMP3, p27^{Kip1}, p57, Bim. MiR-221/222 overexpression induces cell proliferation through the activation of cell cycle and the Akt pathway and blocks TRAIL-induced apoptosis. Moreover, miR-221/222 determine fulvestrant resistance through the activation of the β-catenin pathway.

Fig. (3). MiR-221/222 as tumor suppressor miRs

MiR-221/222 act as tumor suppressor miRs in the erythropoietic lineage cells, and oral tongue squamous cells by targeting c-Kit, matrix metalloproteinase 1 (MMP1) and manganese superoxide dismutase (SOD2).

Table 1

miR-221/222 as oncogenes

miRNA Target	Deregulation in Cancer	Ref.
P27/Kip1	Giloblastoma, thyroid papillary carcinomas, hepatocellular carcinoma, breast, prostate and pancreatic cancer	[21–25]
P57	Hepatocellular carcinoma	[26]
Puma	Glioblastoma	[19]
ER- α FOXO3	Breast	[28, 29]
PTEN TIMP3	NSCLC, Hepatocarcinoma, giloma, gastric cancer	[44]
DDIT4	Hepatocellular carcinoma	[52]
Bim	Prostate cancer	[30]

Table 2

miR-221/222 as Tumor Suppressor Genes

miRNA Target	Deregulation in Cancer	Ref.
SODI MMP1	toncue squamous cell carcinoma (OTSCC)	31
c-Kit	erythrocytes	32