Effects of MIR143 on rat sarcoma signaling networks in solid tumors: A brief overview

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Abstract
Rat sarcoma (RAS) is a well-known oncogene that plays important roles in cancer proliferation, cell survival and cell invasion. RAS exists as three major isoforms, Kirsten rat sarcoma (KRAS), Harvey rat sarcoma (HRAS) and neuroblastoma rat sarcoma (NRAS). Mutations of these genes account for approximately 30% of all cancers. Among them, KRAS mutations are the most common, responsible for 85%, followed by NRAS (12%) and HRAS (3%). Although the development of RAS inhibitors has been explored for over the past decade, so far, no effective inhibitor has been found.

MicroRNA (miRNA) are a class of small non–coding RNA that control the gene expression of pleural target genes at the post–transcriptional level. MiRNA play critical roles in the physiological and pathological processes at work in cancers, such as cell proliferation, cell death, cell invasion and metastasis. MicroRNA-143 (MIR143) is known to function as a tumor suppressor in a variety of cancers. One of its known mechanisms is suppression of RAS expression and its effector signaling pathways, such as PI3K/AKT and MAPK/ERK. Within the last five years, we developed a potent chemically modified MIR143-3p that enabled us to elucidate the details of the KRAS signaling networks at play in colon and other cancer cells. In this review, we will discuss the role of MIR143-3p in those RAS signaling networks that are related to various biological processes of cancer cells. In addition, we will discuss the possibility of the use of MIR143 as a therapeutic drug for targeting RAS signaling networks.

KEYWORDS
AKT, ERK, KRAS positive circuit, MAPK, MIR143, PI3K, RAS

1 | INTRODUCTION

MicroRNA (miRNA) are endogenous small non–coding RNA molecules that have been recognized as key players in carcinogenesis, cell proliferation, differentiation and migration. For the last ten years, microRNA have attracted attention because of their functions as predictive and prognostic markers and their potential as a therapeutic agent.

MicroRNA function as post–transcriptional regulators by binding to the 3′UTR of the target mRNA, whereby they repress protein expression. Each microRNA targets multiple genes or pathways to regulate at the post–transcriptional levels. Consequently, certain miRNA associate with the tumorigenesis of most cancers and
regulate the biological processes, such as cell proliferation, metastasis and cell death in cancer cells.\textsuperscript{5}

MicroRNA that suppress the tumor suppressor gene promote cancer progression and are called onco-miR.\textsuperscript{6,7} In contrast, miRNA that inhibit the oncogenes are called tumor suppressor miRNA. MIR143-3p is one of the best known of the tumor suppressor miRNA. It is down-regulated in various human cancers, such as breast cancer,\textsuperscript{8} gastric cancer,\textsuperscript{9} colorectal cancer,\textsuperscript{10} bladder cancer\textsuperscript{11} and renal cell cancer.\textsuperscript{12} From previous studies, we learned that MIR143-3p functions as a tumor suppressor by modulating rat sarcoma (RAS) signaling networks, which are critical for cell proliferation, cell growth and apoptosis.

In this review, we discuss the roles of MIR143, mainly MIR143-3p, in RAS signaling networks in various cancers and its potential as a therapeutic agent.

2 | FUNCTIONS OF MIR143

MIR143 is located at chromosome 5q32 and the expression of MIR143 is regulated by p53 protein and hypoxia inducible factor-1α (HIF-1α).\textsuperscript{13} MIR143 is co-transcribed with MIR145 in the same primary miRNA, and is thereby downregulated in the same fashion in various kinds of cancers.\textsuperscript{14} MIR143-3p (the guide strand) and MIR143-5p (the passenger strand) are the two isoforms of MIR143, with MIR143-3p being a common isoform in normal tissues. In normal cells, it is highly expressed in mesenchymal cells, such as fibroblasts and smooth muscle cells.\textsuperscript{15} Despite the existence of a few reports regarding the suppressive function of MIR143-5p, knowledge on the function of MIR143-5p remains limited.\textsuperscript{16}

FIGURE 1  Signaling pathways of rat sarcoma (RAS) in cancer. RAS transduces various stimulations from the cell surface to effector signaling pathways, such as PI3K/AKT, MAPK and Ral/GEF pathways.

3 | THE ASSOCIATION OF RAS SIGNALING NETWORKS WITH CANCER AND MICRORNA REGULATING RAT SARCOMA

The RAS gene is one of the best understood of the oncogenes. Its main function is to transduce diverse signals from the cell membrane to the downstream effector signaling pathways related to the proliferation of cancer cells.\textsuperscript{17}

Rat sarcoma occurs in activated and inactivated forms. RAS is inactivated when coupled with GDP, and is activated when it bound to GTP by guanine nucleotide exchange factors (GEF; Figure 1). In contrast, the activated RAS becomes inactivated by GTPase-activating proteins (GAP).\textsuperscript{18} Continuous activation of RAS is mainly caused by gene mutations. Amplification and overexpression of RAS or upregulated stimulation from tyrosine kinase receptors such as EGFR and HER2 result in the continuous activation of RAS in cancer cells.\textsuperscript{19,20} This dysregulation results in the stimulation of its effectors, MAPK/ERK, PI3K/AKT and RalGGEF/RAL signaling pathways, which are the crucial pathways for tumorigenesis, cell proliferation, survival of cancer cells, differentiation and cell invasion.\textsuperscript{21,22}

Rat sarcoma is categorized into three major isoforms: Kirsten rat sarcoma (KRAS), Harvey rat sarcoma (HRAS) and neuroblastoma rat sarcoma (NRAS). KRAS is further sub-classified into KRAS 4A and KRAS 4B.\textsuperscript{23} Dysregulation of these RAS isoforms leads to the aberrant activation of downstream effector signaling pathways in various cancers, and their mutation rates found in human cancers differ among these isoforms. Approximately 98% of RAS mutations are observed at codons 12, 13 or 61. The frequency of mutation site differs by tumor type. KRAS mutations are most observed at codon 12 (72.4%-83%), whereas NRAS most frequently harbors a mutation...
Mutations of these isoform genes are observed in approximately 30% of human cancers; and in those cancers, KRAS is the most frequently altered, accounting for 63%-85%, followed by 12%-25% for NRAS and 3%-12% for HRAS.25,26 There are several microRNA that regulate RAS expression. In 2005, MIRLET7 became the first tumor suppressive miRNA reported to downregulate RAS expression in lung tumors.27 Later, numerous other miRNA were discovered to target RAS or its effector molecules in numerous cancers.28 There are several miRNA reported to target KRAS other than MIRLET7.29 MIR96, MIR126 and MIR206 were downregulated in pancreatic cancer and functioned as tumor suppressors.30-32 Regarding gastrointestinal cancers, MIR27A targets KRAS in esophageal cancer,33 MIR181C34 and MIR43335 target KRAS in GC, and MIR30B targets KRAS in colorectal cancer.36 For breast cancer, MIR30C and MIR200C target KRAS and exhibit anti–tumor effects.37,38 MIR200C functions as a tumor suppressor in lung cancer by negatively regulating KRAS expression.38 However, these miRNA do not decrease the expression levels of KRAS-related SOS1, AKT and ERK, as they are silenced MIR143-3p. Importantly, p53 functions as a transcription factor of MIR143.39 Thus, MIR143-3p could be a major miRNA against RAS networks because of its contribution to the p53/MIR143/RAS cascade.

4 | THE ROLE OF MIR143-3P ON RAT SARCOMA SIGNALING NETWORKS IN VARIOUS CANCERS

Regarding RAS signaling networks, MIR143-3p exerts its anti-tumor effects in various human cancers by targeting RAS and its effector signaling genes, such as AKT and ERK12,40-42 (Figure 2, Table 1). MAPK/ERK pathways and PI3K/AKT pathways are critical pathways for the survival and proliferation of cancer cells. Therefore, the regulation of these pathways is a very important strategy for treating RAS-mutant cancers. Therefore, in this section, we introduce the role of MIR143-3p on RAS signaling networks.

4.1 | Pancreatic cancer

Pancreatic cancer (PC) is a very aggressive cancer with a poor prognosis, and, as mentioned above, a KRAS mutation is frequently observed in PC.41,43 The downregulation of MIR143-3p results in the activation of KRAS signaling networks, which implies an association between MIR143-3p and RAS in PC cells.41 Hu et al showed that the ectopic expression of MIR143 reduces the expression level of KRAS in PC cell lines and, consequently, inhibits the cell invasion, migration and metastasis.44 Kent et al45 showed that a KRAS mutation in PC cells suppresses the expression levels of MIR143/145 and consequently, inhibits the cell invasion, migration and metastasis.44 They demonstrated that the inhibition of KRAS led to the low activity of RhoA, which is associated with tumour migration and invasion. Hu et al also demonstrated the anti-tumor effect of MIR143-3p in a xenograft tumor model of PC cancer cells.44 Kent et al45 showed that a KRAS mutation in PC cells suppresses the expression levels of MIR143/145 through the inhibition of Ras-responsive element-binding protein (RREB1).

4.2 | Gastrointestinal cancer

Colorectal cancer is one of the four major causes of cancer death.46 The growth-suppressive effect of MIR143-3p in colorectal cancer cells by targeting KRAS was first reported in 2009.47 Chen et al47 demonstrated that MIR143-3p inhibits colorectal cancer
cell proliferation in vitro and in vivo by silencing KRAS expression. They validated the growth-suppressive function of MIR143-3p by silencing KRAS, which led to the stimulation of cell proliferation. In addition, they found that the downregulation of KRAS led to the inhibition of phosphorylation of ERK1/2 in colorectal cancer cells. Luo et al. reported that restoration of MIR143-3p expression through use of an MIR143-3p-bearing vector demonstrated an anti-tumor effect in colorectal cancer cell lines. Pagliuca reported that overexpression of MIR143-3p and MIR145 reduces tumor growth both in vitro and in vivo and that the anti-tumor effect was mediated by profound inactivation of the MAPK/ERK signaling pathway.

MIR143 is also associated with the chemosensitivity of colon cancer cells. Fei et al. reported that transfection with MIR143-3p increases the sensitivity to paclitaxel in a KRAS mutant cell line. We reported the synergistic effect of MIR143-3p with an epidermal growth factor receptor (EGFR) inhibitor, cetuximab, in KRAS mutant colon cancer cells; it was demonstrated that MIR143-3p systematically suppresses KRAS signaling networks, including MAPK/ERK and PI3K/AKT, and synergizes with cetuximab for growth inhibition both in vitro and in vivo. In agreement, Gomes et al. reported that ectopic expression of MIR143-3p and MIR-145 increases the sensitivity to cetuximab in both KRAS wild-type and KRAS mutant colon cancer cells. They demonstrated that MIR143-3p exhibits its anti-tumor effect through cetuximab-mediated antibody-dependent cellular cytotoxicity (ADCC) in colon cancer cells.

Recently, we found that the expression level of MIR143-3p is also downregulated in HER2-positive GC cells and that ectopic expression of MIR143 induced cell growth suppression in those cells.

**TABLE 1** The functions of MIR143 in variety types of cancers

| Types of cancer | Target genes | Functions | Reference | Genomic location of miRNA-target interaction |
|----------------|--------------|-----------|-----------|---------------------------------------------|
| Pancreatic cancer | KRAS         | Inhibition of cell invasion and migration | 44 | <Genomic location of MIR143³> chromosome 5:149428918-149429023 |
| | KRAS, RREB1  | Inhibition of tumor growth through Ras-responsive element-binding protein (RREB1) | 45 | |
| Colorectal cancer | KRAS         | Inhibition of cancer cell growth | 47 | <Location of target interaction> |
| | KRAS         | Inhibition of cancer cell proliferation | 48 | KRAS 3’UTR: 109-115 |
| | KRAS         | Inhibition of tumor growth | 49 | RREB1 3’UTR: 1528-1534 |
| | KRAS         | Inhibition of cell growth and enhancement of sensitivity to cetuximab | 42 | AKT 3’UTR: 1029-1035 |
| | KRAS         | Increasing the sensitivity to paclitaxel | 50 | ERK (MAPK1) 3’UTR: 1059-1065 |
| | KRAS         | Enhancement of sensitivity to cetuximab and antibody-dependent cellular cytotoxicity (ADCC) | 51 | SOS1 3’UTR: 3438-3444 |
| |              | Downregulation of expression levels of HER2 | 52 | ERK5 3’UTR: 120-127 |
| Gastric cancer    | KRAS         | Inhibition of tumor growth | 53 | NRAS 3’UTR: 3080-3086 |
| Lung cancer       | KRAS         | Suppression of cancer progression through TGF-β | 40 | |
|                 | EGFR, AKT, ERK | Inhibition of cell proliferation | 53 | |
| Renal cell carcinoma | KRAS       | Suppression of tumor growth | 12 | |
| Bladder cancer    | KRAS, SOS1, AKT, ERK | Inhibition of tumor growth | 54 | |
| | AKT, ERK     | Inhibition of cell growth | 11 | |
| | KRAS         | Inhibition of cell growth. Negative regulation of Musashi-2 | 55 | |
| Prostate cancer   | KRAS         | Enhancement of chemosensitivity to docetaxel | 56 | |
| Laryngeal squamous cell carcinoma | KRAS   | Inhibition of tumor growth | 57 | |
| Nasopharyngeal cancer | KRAS | Suppression of cell growth | 58 | |
| Cervical cancer   | ERK5         | The inhibition of cell proliferation | 59 | |
| Breast cancer     | KRAS         | Inhibition of cell invasion and metastasis | 61 | |
| | ERK5         | Inhibition of epithelial mesenchymal transition (EMT) | 8 | |
| | AKT          | Inhibition of cell proliferation | 62 | |
| Endometrial cancer| MAPK1 (ERK)  | Inhibition of cell invasion and cell migration | 63 | |
| Brain tumor       | NRAS         | Suppression of tumor growth. Increasing chemosensitivity to temozolomide | 65 | |

³Genomic location of MIR143 was presented according to Ensemble Release 98 (September 2019).
same study we demonstrated that MIR143-3p targets KRAS effector signaling genes such as KRAS, AKT and ERK, leading to suppression of GC cell growth, and clarified that MIR143-3p indirectly silences human epidermal growth factor receptor 2 (HER2), resulting in suppression of KRAS activation and its effector signaling pathways.

### 4.3 | Lung cancer

Lung cancer is the leading cause of cancer-related deaths in both males and females worldwide. Chen et al identified the suppressive effect of MIR143-3p in non–small cell lung cancer cell progression. They also demonstrated that MIR143 expression is modulated by TGF-β and that MIR143-3p suppresses lung cancer progression through the silencing of KRAS. EGFR is highly expressed in over 60% of non–small cell lung cancers (NSCLC), implying that EGFR, RAS, MAPK and PI3K/AKT signaling pathways are potential therapeutic targets for NSCLC. Dong and Hu demonstrated the cell growth suppression of exogenous MIR143 through the downregulation of the expression levels of EGFR, AKT and ERK1/2 and the phosphorylation of EGFR, AKT and ERK1/2 in NSCLC in vitro. They also found that the overexpression of MIR143 resulted in the promotion of the apoptosis of lung cancer cells.

### 4.4 | Urinary system cancer

We demonstrated that MIR143-3p silences KRAS and its effector molecules, such as AKT and ERK, in renal cell carcinoma and bladder cancer cells both in vitro and in vivo. Yoshikawa et al demonstrated that the ectopic expression of MIR143 led to the downregulation of the expression levels of SOS1, which switches inactivated RAS to the activated RAS, KRAS, AKT and ERK. Noguchi et al demonstrated the combination therapy with the ectopic expression of MIR143 and MIR145 showed the synergistic inhibition of bladder cancer cells through modulating PI3K/AKT and MAPK signaling pathways. Recently, we discovered that MIR143-3p negatively regulated the KRAS/Musashi-2 cascade and, consequently, suppressed the cell growth in bladder cancer through downregulation of the KRAS protein expression level at the translational step. Xu et al demonstrated that MIR143 overexpression inhibits KRAS expression and its effector MAPK/ERK signaling pathway, which results in enhanced chemosensitivity to docetaxel in prostate cancer.

### 4.5 | Head and neck cancer

Zhang et al reported that MIR143-3p exhibits an anti–tumor effect on laryngeal squamous cell carcinoma, the most frequent cancer of the head and neck cancers, by targeting KRAS and its effector MAPK/ERK signaling pathway. In addition, Xu’s group demonstrated that the MIR143 overexpression inhibits the cell growth of nasopharyngeal cancer both in vitro and in vivo. Zheng et al demonstrated that the overexpression of MIR143 suppressed the cell proliferation of cervical cancer cells in vitro. They found that MIR143-3p negatively regulated ERK5 expression, which led to the suppression of cervical cancer cell lines.

### 4.6 | Breast cancer

Even though KRAS mutations are detected in <5% of breast cancer patients, the mutation rate is reported to be 10%-12% in the case of metastatic breast cancer. The ectopic expression of MIR143 inhibits cell growth and cell invasion by silencing KRAS and the epithelial–mesenchymal transition, which is one of the key processes involved in metastasis. The MAPK/ERK signaling pathway, which is the effector signaling pathway of RAS, plays a critical role in metastasis as well as cell proliferation. ERK5 is the essential molecule for the activation of MAPK/ERK signaling pathways. MIR143-3p strongly suppressed the function of ERK5, which modulated a key molecule for EMT, and, consequently, led to the inhibition of metastasis in breast cancer. Garcia Vazquez et al reported that ectopic restoration of MIR143 inhibited cell proliferation through targeting AKT and its phosphorylation in breast cancer cells.

### 4.7 | Female genital system tumors

As mentioned above, the MAPK/ERK signaling pathway is a key signaling pathway of RAS networks, which play a role in metastasis. In endometrial cancer (EC), the downregulation of MAPK1 was observed with the ectopic expression of MIR143 and the suppression of invasion and migration was observed.

### 4.8 | Brain tumor

Glioma is an aggressive type of brain tumor with a poor prognosis. Wang et al demonstrated that the overexpression of MIR143 inhibits the tumor growth of glioma in vitro and in vivo through silencing NRAS. In addition, they demonstrated that the restoration of MIR143 enhances the chemosensitivity to temozolomide, which is the standard initial drug for the treatment of glioma.

### 5 | Future Perspectives: CM-MIR143-3P as a Candidate of Therapeutic Agents That Target Rat Sarcoma Signaling Networks

The aberrant activation of PI3K/AKT and MAPK/ERK signaling pathways is often seen in many kinds of cancers, and many therapeutic agents targeting molecules related to these pathways have been developed. The regulation of these pathways is a very important strategy for treating RAS-mutant cancers. In fact,
numerous inhibitors targeting these pathways have been clinically evaluated. For instance, for the MAPK pathway, the inhibitors for RAF, MEK and ERK have been clinically evaluated, as well as the inhibitors for the PI3K/AKT pathway. However, the challenge of suppressing RAS-driven cancers remains. Global suppression of these signaling pathways is crucial for a durable effect on cancers, because inhibition of only a single pathway will result in resistance to the agent through activation of alternative signaling pathways. For instance, a PI3K inhibitor will cause the upregulation of HER2 or HER3, which induces the upregulation of the MAPK signaling pathway in breast cancer. In addition, a MEK inhibitor will lead to the upregulation of the PI3K/AKT pathway in colorectal cancer. For these reasons, another strategy to attack RAS-driven cancers is necessary. One potential approach is to downregulate KRAS expression with small interfering RNA or microRNA. Numerous microRNA, such as MIRLET7 and MIR200C, have been reported in preclinical models to function as tumor suppressors in various types of cancer. However, these microRNA cannot possess the function to suppress KRAS networks systematically. Therefore, we developed and reported on a chemically modified MIR143-3p (CM-MIR143-3p). We developed more than 120 different MIR143 derivatives for two main reasons. The first reason is to make MIR143 RNase resistant. The other reason is to enhance the ability to bind the target genes of MIR143 and the ability to suppress the translation of those genes. Among the derivatives, CM-MIR143-3p, which is chemically modified only in the passenger strand (Figure 3), showed strikingly potent anti-proliferative activity. The CM-MIR143-3p systemically inhibits the KRAS signaling networks, which include RAS-MAPK and PI3K-AKT effector signaling pathways, by silencing KRAS, SOS1, AKT and ERK both in vitro and in vivo in colon cancer cells. In the same study, we further demonstrated the synergistic effect of an EGFR inhibitor and CM-MIR143-3p in KRAS mutant cells. In line with this result, Gomes et al. reported that MIR143 increases the sensitivity to cetuximab in both KRAS wild-type and KRAS mutant colon cancer cells. These results suggest the potential combinational therapy of MIR143 and EGFR inhibitors. Other than colorectal cancer, MIR143-3p displayed tumor suppressor effects on various types of cancers, such as PC, head and neck cancers, lung cancers and breast cancers. Our group also found that CM-MIR143-3p exerts efficient suppression of KRAS networks in renal cell cancer and bladder cancer by systematically suppressing the KRAS networks, including PI3K/AKT and MAPK/ERK pathways. Importantly, we demonstrated that the silencing of KRAS alone induces a "positive circuit" of KRAS expression; that is, the downregulation of KRAS is transient, finally leading to re-upregulation of KRAS expression, because KRAS is one of the target genes of each KRAS effector signaling pathway (Figure 2). Thus, CM-MIR143-3p enabled us to understand the detailed networks and KRAS positive circuit in KRAS-driven colon cancers. Despite the remarkable effects of CM-MIR143-3p, there is a limitation to its use as a therapeutic agent in clinical practice. The main issue is the mode of its delivery to the target organs. A stable mode of delivery of microRNA is essential, because microRNA is degraded by RNA nuclease, which exists in abundance throughout the circulation. Thus, virus and non–virus methods are being developed as delivery vehicles. Retrovirus, adenovirus, aden-associated virus and herpes virus have been used as vectors for overexpressing or suppressing microRNA. Non–viral delivery vehicles that are positively charged form a complex with the negatively charged microRNA or other nucleic acids. In this review, we discussed the anti-cancer effects of MIR143-3p on different types of cancers, with suppression realized by targeting the genes related to RAS signaling networks. Recent translational research has revealed the importance of suppressing multiple signaling pathways to treat cancers. Our CM-MIR143-3p has enabled us to understand the detailed signaling networks of KRAS and related signaling cascades. Given the critical effects of CM-MIR143-3p in suppressing the expression of both RAS and its effector signaling genes, we cannot help but speculate that CM-MIR143-3p efficiently functions as a therapeutic agent to impair the KRAS signaling networks. This work was performed with the support of SHIONOGI. The authors declare no conflict of interest.

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