REVIEW ARTICLE

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

Yoshihisa Tokumaru[1,2] | Kazuaki Takabe[1,3] | Kazuhiro Yoshida[2] |
Yukihiro Akao[4]

[1]Breast Surgery, Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, New York

[2]Department of Surgical Oncology, Graduate School of Medicine, Gifu University, Gifu, Japan

[3]Department of Surgery, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, The State University of New York, Buffalo, New York

[4]United Graduate School of Drug and Medical Information Sciences, Gifu University, Gifu, Japan

Correspondence
Yukihiro Akao, United Graduate School of Drug and Medical Information Sciences, Gifu University, Gifu, Japan.
Email: yakao@gifu-u.ac.jp

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.[1] 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.[2,3]

MicroRNA function as post–transcriptional regulators by binding to the 3′UTR of the target mRNA, whereby they repress protein expression.[4] 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.[5]

MicroRNA that suppress the tumor suppressor gene promote cancer progression and are called onco-miR.[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,[8] gastric cancer (GC),[9] colorectal cancer,[10] bladder cancer[11] and renal cell cancer.[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).[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.[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.[15] Despite the existence of a few reports regarding the suppressive function of MIR143-5p, knowledge on the function of MIR143-5p remains limited.[16]

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.[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).[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.[19,20] This dysregulation results in the stimulation of its effectors, MAPK/ERK, PI3K/AKT and RalGEF/RAL signaling pathways, which are the crucial pathways for tumorigenesis, cell proliferation, survival of cancer cells, differentiation and cell invasion.[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.[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
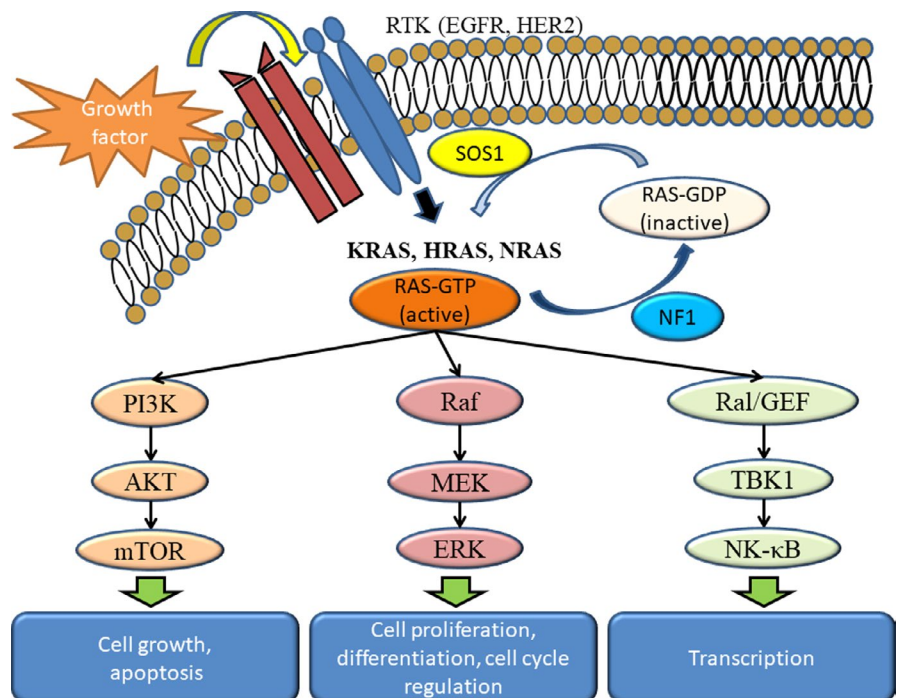

FIGURE 1 Signaling pathways of rat sarcoma (RAS) in cancer. RAS transduces various stimulations from the cell surface to effector signaling pathways, such as PI3K/AKT, MAPK and Ral/GEF pathways
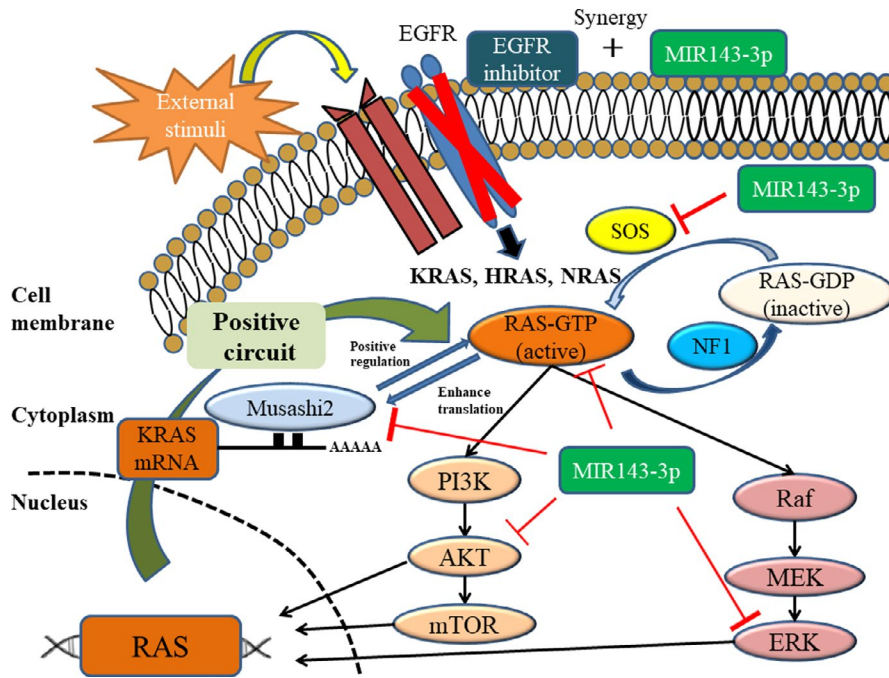
FIGURE 2 The suppressive role of MIR143-3p in rat sarcoma (RAS) signaling pathway. MIR143-3p targets RAS, AKT, ERK and Musashi2 and exhibits synergic effect with EGFR inhibitor. In addition, the schema demonstrates "positive circuit" of KRAS expression

at codon 61 (63%-68.5%).[24,25] 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] MIR181C [34] and MIR433 [35] 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 ERK [12,40-42] (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 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] They demonstrated that the inhibition of KRAS led to the low activity of RhoA, which is associated with tumor 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 al[45] 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 al[47] demonstrated that MIR143-3p inhibits colorectal cancer

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 *MIR143*[a]> 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 ERK (MAPK1) 3′UTR: 1059-1065
	KRAS	Increasing the sensitivity to paclitaxel	50	SOS1 3′UTR: 3438-3444
	KRAS	Enhancement of sensitivity to cetuximab and antibody-dependent cellular cytotoxicity (ADCC)	51	ERK5 3′UTR: 120-127 NRAS 3′UTR: 3080-3086
Gastric cancer	KRAS	Inhibition of tumor growth. Downregulation of expression levels of HER2	52	
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	

[a]Genomic location of *MIR143* was presented according to Ensemble Release 98 (September 2019).

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[48] 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.[49]

MIR143 is also associated with the chemosensitivity of colon cancer cells. Fei et al[50] revealed 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.[42] In agreement, Gomes et al[51] 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 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.[52] In that

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.[52]

4.3 | Lung cancer

Lung cancer is the leading cause of cancer-related deaths in both males and females worldwide.[46] 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.[40] 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.[53] 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.[53]

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.[12] 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.[54] 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.[11] 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.[55] Xu et al[56] 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[57] 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.[58] Zheng et al

demonstrated that the overexpression of MIR143 suppressed the cell proliferation of cervical cancer cells in vitro.[59] 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.[60] 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.[61] 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.[8] 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.[8] Garcia Vazquez et al reported that ectopic restoration of MIR143 inhibited cell proliferation through targeting AKT and its phosphorylation in breast cancer cells.[62]

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.[63]
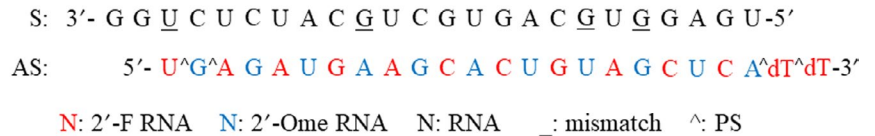
4.8 | Brain tumor

Glioma is an aggressive type of brain tumor with a poor prognosis.[64] Wang et al[65] 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.[65]

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.[66,67] The regulation of these pathways is a very important strategy for treating RAS-mutant cancers. In fact,

FIGURE 3 Sequence of CM-MIR143-3p and its chemical modification. F RNA, Fluoro-RNA; Ome RNA, O-Methyl RNA; PS, phosphorothioate

CM-MIR143-3p

S: 3'- G G U C U C U A C G U C G U G A C G U G G A G U -5'

AS: 5'- U^G^A G A U G A A G C A C U G U A G C U C A^dT^dT -3'

N: 2'-F RNA   N: 2'-Ome RNA   N: RNA   _: mismatch   ^: PS

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.[26,68] 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.[69,70] In addition, a MEK inhibitor will lead to the upregulation of the PI3K/AKT pathway in colorectal cancer.[71] 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 miRNA. Numerous microRNA, such as MIRLET7 and MIR200C, have been reported in preclinical models to function as tumor suppressors in various types of cancer.[27,38] However, these miRNA 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.[42] In the same study, we further demonstrated the synergistic effect of an EGFR inhibitor and CM-MIR143-3p in KRAS mutant cells.[51] In line with this result, Gomes et al[51] 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.[45,53,58,62] Our group also found that CM-MIR143-3p exerts efficient suppression of KRAS networks in renal cell cancer [12] and bladder cancer [54] 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 miRNA is essential, because miRNA 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, adeno-associated virus and herpes virus have been used as vectors for overexpressing or suppressing miRNA.[72] Non–viral delivery vehicles that are positively charged form a complex with the negatively charged miRNA or other nucleic acids.[73]

6 | CONCLUSION

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.

DISCLOSURE

The authors declare no conflict of interest.

ORCID

Yoshihisa Tokumaru https://orcid.org/0000-0003-2481-7739
Kazuaki Takabe https://orcid.org/0000-0002-6435-4241
Kazuhiro Yoshida https://orcid.org/0000-0003-1408-0104
Yukihiro Akao https://orcid.org/0000-0001-8902-5367

REFERENCES

1. Drusco A, Croce CM. MicroRNAs and cancer: a long story for short RNAs. *Adv Cancer Res.* 2017;135:1-24.

2. Beg MS, Brenner AJ, Sachdev J, et al. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Investig New Drugs*. 2017;35:180-188.

3. Kawaguchi T, Yan L, Qi Q, et al. Novel MicroRNA-based risk score identified by integrated analyses to predict metastasis and poor prognosis in breast cancer. *Ann Surg Oncol*. 2018;25:4037-4046.

4. Croce CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet*. 2009;10:704-714.

5. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. *Annu Rev Pathol*. 2014;9:287-314.

6. Kawaguchi T, Yan L, Qi Q, et al. Overexpression of suppressive microRNAs, miR-30a and miR-200c are associated with improved survival of breast cancer patients. *Sci Rep*. 2017;7(1):15945.

7. Oliveto S, Mancino M, Manfrini N, Biffo S. Role of microRNAs in translation regulation and cancer. *World J Biol Chem*. 2017;8:45-56.

8. Zhai L, Ma C, Li W, Yang S, Liu Z. miR-143 suppresses epithelial-mesenchymal transition and inhibits tumor growth of breast cancer through down-regulation of ERK5. *Mol Carcinog*. 2016;55:1990-2000.

9. Zhang Q, Feng Y, Liu P, Yang J. MiR-143 inhibits cell proliferation and invasion by targeting DNMT3A in gastric cancer. *Tumour Biol*. 2017;39(7):1010428317711312. https://doi.org/10.1177/10104 28317711312

10. Ak S, Tunca B, Tezcan G, et al. MicroRNA expression patterns of tumors in early-onset colorectal cancer patients. *J Surg Res*. 2014;191:113-122.

11. Noguchi S, Yasui Y, Iwasaki J, et al. Replacement treatment with microRNA-143 and -145 induces synergistic inhibition of the growth of human bladder cancer cells by regulating PI3K/Akt and MAPK signaling pathways. *Cancer Lett*. 2013;328:353-361.

12. Takai T, Tsujino T, Yoshikawa Y, et al. Synthetic miR-143 exhibited an anti–cancer effect via the downregulation of K-RAS networks of renal cell cancer cells in vitro and in vivo. *Mol Ther*. 2019;27:1017-1027.

13. Karimi L, Mansoori B, Shanebandi D, Mohammadi A, Aghapour M, Baradaran B. Function of microRNA-143 in different signal pathways in cancer: new insights into cancer therapy. *Biomed Pharmacother*. 2017;91:121-131.

14. Iio A, Nakagawa Y, Hirata I, Naoe T, Akao Y. Identification of non–coding RNAs embracing microRNA-143/145 cluster. *Mol Cancer*. 2010;9:136.

15. Kent OA, McCall MN, Cornish TC, Halushka MK. Lessons from miR-143/145: the importance of cell-type localization of miRNAs. *Nucleic Acids Res*. 2014;42:7528-7538.

16. Sanada H, Seki N, Mizuno K, et al. Involvement of dual strands of miR-143 (miR-143-5p and miR-143-3p) and their target oncogenes in the molecular pathogenesis of lung adenocarcinoma. *Int J Mol Sci*. 2019;20:4482.

17. Haigis KM. KRAS alleles: the devil is in the detail. *Trends Cancer*. 2017;3:686-697.

18. Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. *Cell*. 2017;170:17-33.

19. Valtorta E, Misale S, Sartore-Bianchi A, et al. KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy. *Int J Cancer*. 2013;133:1259-1265.

20. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodríguez Yoldi MJ. Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. *Int J Mol Sci*. 2017;18:197.

21. Martin TD, Chen XW, Kaplan RE, et al. Ral and Rheb GTPase activating proteins integrate mTOR and GTPase signaling in aging, autophagy, and tumor cell invasion. *Mol Cell*. 2014;53:209-220.

22. Temraz S, Mukherji D, Shamseddine A. Dual inhibition of MEK and PI3K pathway in KRAS and BRAF mutated colorectal cancers. *Int J Mol Sci*. 2015;16:22976-22988.

23. Hobbs GA, Der CJ, Rossman KL. RAS isoforms and mutations in cancer at a glance. *J Cell Sci*. 2016;129:1287-1292.

24. Zhou B, Der CJ, Cox AD. The role of wild type RAS isoforms in cancer. *Semin Cell Dev Biol*. 2016;58:60-69.

25. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2:401-404.

26. Papke B, Der CJ. Drugging RAS: know the enemy. *Science*. 2017;355:1158-1163.

27. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. *Cell*. 2005;120:635-647.

28. Sharma SB, Ruppert JM. MicroRNA-based therapeutic strategies for targeting mutant and wild type RAS in cancer. *Drug Dev Res*. 2015;76:328-342.

29. Masliah-Planchon J, Garinet S, Pasmant E. RAS-MAPK pathway epigenetic activation in cancer: miRNAs in action. *Oncotarget*. 2016;7:38892-38907.

30. Yu S, Lu Z, Liu C, et al. miRNA-96 suppresses KRAS and functions as a tumor suppressor gene in pancreatic cancer. *Cancer Res*. 2010;70:6015-6025.

31. Jiao LR, Frampton AE, Jacob J, et al. MicroRNAs targeting oncogenes are down-regulated in pancreatic malignant transformation from benign tumors. *PLoS ONE*. 2012;7:e32068.

32. Keklikoglou I, Hosaka K, Bender C, et al. MicroRNA-206 functions as a pleiotropic modulator of cell proliferation, invasion and lymphangiogenesis in pancreatic adenocarcinoma by targeting ANXA2 and KRAS genes. *Oncogene*. 2015;34:4867-4878.

33. Zhu L, Wang Z, Fan Q, Wang R, Sun Y. microRNA-27a functions as a tumor suppressor in esophageal squamous cell carcinoma by targeting KRAS. *Oncol Rep*. 2014;31:280-286.

34. Hashimoto Y, Akiyama Y, Otsubo T, Shimada S, Yuasa Y. Involvement of epigenetically silenced microRNA-181c in gastric carcinogenesis. *Carcinogenesis*. 2010;31:777-784.

35. Guo LH, Li H, Wang F, Yu J, He JS. The tumor suppressor roles of miR-433 and miR-127 in gastric cancer. *Int J Mol Sci*. 2013;14:14171-14184.

36. Liao WT, Ye YP, Zhang NJ, et al. MicroRNA-30b functions as a tumour suppressor in human colorectal cancer by targeting KRAS, PIK3CD and BCL2. *J Pathol*. 2014;232:415-427.

37. Tanic M, Yanowsky K, Rodriguez-Antona C, et al. Deregulated miRNAs in hereditary breast cancer revealed a role for miR-30c in regulating KRAS oncogene. *PLoS ONE*. 2012;7:e38847.

38. Kopp F, Wagner E, Roidl A. The proto-oncogene KRAS is targeted by miR-200c. *Oncotarget*. 2014;5:185-195.

39. Cabrita MA, Vanzyl EJ, Hamill JD, et al. A Temperature sensitive variant of p53 Drives p53-dependent MicroRNA expression without evidence of widespread post–transcriptional gene silencing. *PLoS ONE*. 2016;11:e0148529.

40. Cheng T, Hu C, Yang H, Cao L, An J. Transforming growth factor-beta-induced miR143 expression in regulation of non–small cell lung cancer cell viability and invasion capacity in vitro and in vivo. *Int J Oncol*. 2014;45:1977-1988.

41. Karmakar S, Kaushik G, Nimmakayala R, Rachagani S, Ponnusamy MP, Batra SK. MicroRNA regulation of K-Ras in pancreatic cancer and opportunities for therapeutic intervention. *Semin Cancer Biol*. 2019;54:63-71.

42. Akao Y, Kumazaki M, Shinohara H, et al. Impairment of K-Ras signaling networks and increased efficacy of epidermal growth factor receptor inhibitors by a novel synthetic miR-143. *Cancer Sci*. 2018;109:1455-1467.

43. Hawa Z, Haque I, Ghosh A, Banerjee S, Harris L, Banerjee SK. The miRacle in pancreatic cancer by miRNAs: tiny angels or devils in disease progression. *Int J Mol Sci*. 2016;17:809.

44. Hu Y, Ou Y, Wu K, Chen Y, Sun W. miR-143 inhibits the metastasis of pancreatic cancer and an associated signaling pathway. *Tumour Biol*. 2012;33:1863-1870.

45. Kent OA, Fox-Talbot K, Halushka MK. RREB1 repressed miR-143/145 modulates KRAS signaling through downregulation of multiple targets. *Oncogene*. 2013;32:2576-2585.

46. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69:7-34.

47. Chen X, Guo X, Zhang H, et al. Role of miR-143 targeting KRAS in colorectal tumorigenesis. *Oncogene*. 2009;28:1385-1392.

48. Luo Q, Basnet S, Dai Z, Li S, Zhang Z, Ge H. A novel E1B55kDa-deleted oncolytic adenovirus carrying microRNA-143 exerts specific antitumor efficacy on colorectal cancer cells. *Am J Transl Res*. 2016;8:3822-3830.

49. Pagliuca A, Valvo C, Fabrizi E, et al. Analysis of the combined action of miR-143 and miR-145 on oncogenic pathways in colorectal cancer cells reveals a coordinate program of gene repression. *Oncogene*. 2013;32:4806-4813.

50. Fei BY, Wang XY, Fang XD. MicroRNA-143 replenishment re-sensitizes colorectal cancer cells harboring mutant, but not wild-type, KRAS to paclitaxel treatment. *Tumour Biol*. 2016;37:5829-5835.

51. Gomes SE, Simoes AE, Pereira DM, Castro RE, Rodrigues CM, Borralho PM. miR-143 or miR-145 overexpression increases cetuximab-mediated antibody-dependent cellular cytotoxicity in human colon cancer cells. *Oncotarget*. 2016;7:9368-9387.

52. Tokumaru Y, Tajirika T, Sugito N, et al. Synthetic miR-143 inhibits growth of HER2-positive gastric cancer cells by suppressing KRAS networks including DDX6 RNA helicase. *Int J Mol Sci*. 2019;20:1697.

53. Dong YZ, Hu T. Effects of miR-143 overexpression on proliferation, apoptosis, EGFR and downstream signaling pathways in PC9/GR cell line. *Eur Rev Med Pharmacol Sci*. 2018;22:1709-1716.

54. Yoshikawa Y, Taniguchi K, Tsujino T, et al. Anti–cancer effects of a chemically modified miR-143 on bladder cancer by either systemic or intravesical treatment. *Mol Ther Method Clin Dev*. 2019;13:290-302.

55. Tsujino T, Sugito N, Taniguchi K, et al. MicroRNA-143/Musashi-2/KRAS cascade contributes positively to carcinogenesis in human bladder cancer. *Cancer Sci*. 2019;110:2189-2199.

56. Xu B, Niu X, Zhang X, et al. miR-143 decreases prostate cancer cells proliferation and migration and enhances their sensitivity to docetaxel through suppression of KRAS. *Mol Cell Biochem*. 2011;350:207-213.

57. Zhang F, Cao H. MicroRNA1433p suppresses cell growth and invasion in laryngeal squamous cell carcinoma via targeting the kRas/Raf/MEK/ERK signaling pathway. *Int J Oncol*. 2019;54:689-701.

58. Xu YF, Li YQ, Guo R, et al. Identification of miR-143 as a tumour suppressor in nasopharyngeal carcinoma based on microRNA expression profiling. *Int J Biochem Cell Biol*. 2015;61:120-128.

59. Zheng F, Zhang J, Luo S, et al. miR-143 is associated with proliferation and apoptosis involving ERK5 in HeLa cells. *Oncol Lett*. 2016;12:3021-3027.

60. Cejalvo JM, Perez-Fidalgo JA, Ribas G, et al. Clinical implications of routine genomic mutation sequencing in PIK3CA/AKT1 and KRAS/

61. NRAS/BRAF in metastatic breast cancer. *Breast Cancer Res Treat*. 2016;160:69-77.

61. Tavanafar F, Safaralizadeh R, Hosseinpour-Feizi MA, et al. Restoration of miR-143 expression could inhibit migration and growth of MDA-MB-468 cells through down-regulating the expression of invasion-related factors. *Biomed Pharmacother*. 2017;91:920-924.

62. Garcia-Vazquez R, Marchat LA, Ruiz-Garcia E, et al. MicroRNA-143 is associated with pathological complete response and regulates multiple signaling proteins in breast cancer. *Technol Cancer Res Treat*. 2019;18:84384-84395.

63. Chang L, Zhang D, Shi H, Bian Y, Guo R. MiR-143 inhibits endometrial cancer cell proliferation and metastasis by targeting MAPK1. *Oncotarget*. 2017;8:84384-84395.

64. Seliger C, Hau P. Drug repurposing of metabolic agents in malignant glioma. *Int J Mol Sci*. 2018;19:2768.

65. Wang L, Shi ZM, Jiang CF, et al. MiR-143 acts as a tumor suppressor by targeting N-RAS and enhances temozolomide-induced apoptosis in glioma. *Oncotarget*. 2014;5:5416-5427.

66. Samatar AA, Poulikakos PI. Targeting RAS-ERK signalling in cancer: promises and challenges. *Nat Rev Drug Discov*. 2014;13:928-942.

67. Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X. Targeting PI3K in cancer: mechanisms and advances in clinical trials. *Mol Cancer*. 2019;18:26.

68. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: mission possible? *Nat Rev Drug Discov*. 2014;13:828-851.

69. Serra V, Scaltriti M, Prudkin L, et al. PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. *Oncogene*. 2011;30:2547-2557.

70. Chakrabarty A, Sanchez V, Kuba MG, Rinehart C, Arteaga CL. Feedback upregulation of HER3 (ErbB3) expression and activity attenuates antitumor effect of PI3K inhibitors. *Proc Natl Acad Sci USA*. 2012;109:2718-2723.

71. Balmanno K, Chell SD, Gillings AS, Hayat S, Cook SJ. Intrinsic resistance to the MEK1/2 inhibitor AZD6244 (ARRY-142886) is associated with weak ERK1/2 signalling and/or strong PI3K signalling in colorectal cancer cell lines. *Int J Cancer*. 2009;125:2332-2341.

72. Abba ML, Patil N, Leupold JH, et al. MicroRNAs as novel targets and tools in cancer therapy. *Cancer Lett*. 2017;387:84-94.

73. Hayashi K, Chaya H, Fukushima S, et al. Influence of RNA strand rigidity on polyion complex formation with block cationers. *Macromol Rapid Commun*. 2016;37:486-493.