Extended Abstract Journal of Clinical Medicine and Therapeutics 2020
Vol.5 No.2

Euro Pharmaceutics 2018: Impairment of K-Ras signalling networks and increased efficacy of epidermal growth factor receptor inhibitors by a novel synthetic miR-143- Yukihiro Akao- Gifu University.

Yukihiro Akao[1]

[1]Gifu University , Japan

Abstract:

Despite considerable research on K-Ras inhibitors, none had been established until now. We synthesized nuclease-resistant synthetic miR-143 (miR-143#12), which strongly silenced K-Ras, its effector signal molecules AKT and ERK, and the K-Ras activator Sos1. We examined the anti-proliferative effect of miR-143#12 and the mechanism in human colon cancer DLD-1 cell (G13D) and other cell types harboring K-Ras mutations. Cell growth was markedly suppressed in a concentration-dependent manner by miR-143#12 (IC50: 1.32 nmol L−1) with a decrease in the K-Ras mRNA level. Interestingly, this mRNA level was also downregulated by either a PI3K/AKT or MEK inhibitor, which indicates a positive circuit of K-Ras mRNA expression. MiR-143#12 silenced cytoplasmic K-Ras mRNA expression and impaired the positive circuit by directly targeting AKT and ERK mRNA. Combination treatment with miR-143#12 and a low-dose EGFR inhibitor induced a synergistic inhibition of growth with a marked inactivation of both PI3K/AKT and MAPK/ERK signaling pathways. However, silencing K-Ras by siR-KRas instead of miR-143#12 did not induce this synergism through the combined treatment with the EGFR inhibitor. Thus, miR-143#12 perturbed the K-Ras expression system and K-Ras activation by silencing Sos1 and, resultantly, restored the efficacy of the EGFR inhibitors. The in vivo results also supported those of the in vitro experiments. The extremely potent miR-143#12 enabled us to understand K-Ras signaling networks and shut them down by combination treatment with this miRNA and EGFR inhibitor in K-Ras-driven colon cancer cell lines.

Introduction:

The 3 classical mammalian ras genes, K-ras, N-ras and H-ras, encode 21-Kd proteins that are members of the guanine nucleotide-binding protein superfamily.1, 2 The canonical properties of Ras are those of a small GTPase that normally cycles between a GTP-bound active and a GDP-bound inactive state.This cycle is negatively regulated by GTPase-activating proteins that stimulate the intrinsic GTPase activity and are positively regulated by guanine nucleotide exchange factors (GEF). Ras is normally present in the GDP-bound inactive state, which can be changed to the activated state by extracellular stimuli such as the presence of mitogens, cytokines and growth factors. On activation, Ras exerts its functions through protein-protein interactions with effectors, such as Raf kinase and PI3K, to promote cell growth and survival.

In 1982, mutant Ras genes were detected in human cancers, marking the first discovery of mutated genes in cancer patients. Indeed, Ras mutations are genetic events that have been detected in 30% of all human cancers, with the specific Ras isoform generally differing according to the cancer type.6 Mutations in K-Ras account for approximately 85% of all Ras mutations, those in N-Ras for approximately 15% and those in H-Ras for less than 1%; these are single base missense mutations, mainly in codons 12, 13 or 61 of exons 2 and 3.5, In colon and rectal carcinomas, K-Ras is also the predominantly mutated isoform, whereas N-Ras mutations are infrequent, and H-Ras mutations have not been detected.12 Cancer-causing mutations impair the GTPase activity of Ras, causing Ras to accumulate in the GTP-bound active state, which transmits strong downstream signals

Materials And Methods:

- Cell culture and cell viability
- Assay for stability of miRNA in vitro
- Western blotting
- Cell transfection with miRNA or siRNA
- Quantitative RT-PCR
- K-Ras-GTP assay
- Assay for luciferase activity
- In vivo tumor model and administration of the syn-miR-143
- Statistics

Results:

Growth inhibition by syn-miR-143 of K-Ras mutant human colon cancer DLD-1 (K-RasG13D) cell line

To explore the use of miR-143 as a possible K-Ras inhibitor for K-Ras mutant colon cancer cells, we designed and synthesized some miR-143 having different structures of the double strand for acquiring nuclease resistance. These results indicated that syn-miR-143 are potent growth suppressors at extremely low concentrations and possibly suppressed the expression and activation systems of K-Ras in K-Ras-driven DLD-1 colon cancer cells, which was not observed with Am.

K-Ras effector signaling pathways enhanced the transcription of K-Ras itself

This work is partly presented at 17th Annual Congress on Pharmaceutics & Drug Delivery Systems on September 20-22, 2018 at Prague, Czech Republic

It has been reported that silencing Ras by miR-143 inhibits the growth of Ras mutant human cancer cell lines both in vitro and in vivo.32, 33 When we compared the effects of syn-miR-143 and siR-KRas, the latter being considered to be equal to Ras inhibitors on the cell growth of K-Ras mutant cells that the expression level of K-Ras was decreased by either of 2 different siRNA for K-Ras, which bind to the ORF and 3′UTR regions of K-Ras, respectively, The mRNA level of the control (0; DMSO alone) is indicated as 100%. E and G, Western blot analysis was performed to determine the levels of Ras at 24 h after the treatment. β-actin was used as an internal control

Syn-miR-143 silenced Sos1 by RNAi we show that the ectopic expression of syn-miR-143 decreased the level of K-Ras-GTP. According to in silico prediction tools in TargetScan, Sos1 has an miR-143 binding site in its 3′UTR. To validate Sos1 as a target gene of miR-143, we performed a luciferase reporter assay. Growth inhibition by combined treatment with syn-miR-143 and cetuximab

The effectiveness of cetuximab is now limited to patients with K-Ras wild-type tumors. Above we showed that the ectopic expression of syn-miR-143 significantly decreased the levels of K-Ras mRNA and K-Ras-GTP through perturbation of the positive circuit and activation of K-Ras-GDP

Tumor suppressive effect of syn-miR-143 on in vivo experiment

To further validate the growth inhibitory effect of syn-miR-143, we performed an in vivo study in which control-miR or syn-miR-143 were administrated systemically every 72 hours (750 μg/kg/administration) 4 times to nude mice that had been subcutaneously inoculated with DLD-1 (K-RasG13D) cells

Effects of syn-miR-143 in other K-Ras mutation harboring colon cancer cell lines

To further validate the effects of syn-miR-143 on other K-Ras wild and mutant human colon cancer cell lines, we performed the same experiments by using SW48 (K-RasWild/B-RafWild), HT29 (K-RasWild/B-Raf V600E) and SW480 (K-RasG12V/B-RafWild) cells.

Discussion:

We demonstrated that chemically-modified miR-143#12 exhibited a potent suppressive effect on K-Ras networks and that K-Ras-mutant colon cancer DLD-1 cells established a positive circuit through the constitutive K-Ras activation-stimulation of effector signaling pathways, resulting in enhanced nuclear K-Ras transcription, which was clearly disclosed by using the potent chemically-modified miR-143#12 This positive circuit was also true in the case of another K-Ras mutant, one in SW480 cells The miR-143#12 impaired K-Ras networks including the positive circuit by silencing the key molecules of the networks Furthermore, a well-modified drug delivery system will be required to distribute miR-143#12 into tumors as an RNA medicine against Ras-driven cancers.

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