Regulation of ROCK1/2 by long non-coding RNAs and circular RNAs in different cancer types (Review)

AMMAD AHMAD FAROOQI[1], RABBIA ZAHID[2], HUMAIRA NAUREEN[3], RUKSET ATTAR[4], MARIA GAZOULI[5], ROSSANA BERARDI[6], JOLANTA SZELACHOWSKA[7,8], RAFAŁ MATKOWSKI[7,8] and EDYTA PAWLAK[9]

[1]Department of Molecular Oncology, Institute of Biomedical and Genetic Engineering, Islamabad 54000; [2]Institute of Chemistry, University of Punjab, Lahore 43000; [3]Faculty of Pharmaceutical Sciences, Riphah International University, Islamabad 54000, Pakistan; [4]Department of Obstetrics and Gynecology, Yeditepe University 34280, Turkey; [5]Department of Basic Medical Sciences, Laboratory of Biology, Medical School, National and Kapodistrian University of Athens, Athens 54634, Greece; [6]Oncology Clinic-Marche Polytechnic University, Azienda Ospedaliero-Universitaria Ospedali Riuniti Umberto I-GM Lancisi-G Salesi di Ancona, I-60126 Ancona, Italy; [7]Department of Oncology, Wroclaw Medical University; [8]Wroclaw Comprehensive Cancer Centre, 53-413 Wroclaw; [9]Department of Experimental Therapy, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 50-013 Wroclaw, Poland

Received November 21, 2020; Accepted May 19, 2021

DOI: 10.3892/ol.2022.13279

Abstract. Recent breakthroughs in high-throughput technologies have enabled the development of a better understanding of the functionalities of rho-associated protein kinases (ROCKs) under various physiological and pathological conditions. Since their discovery in the late 1990s, ROCKs have attracted the attention of interdisciplinary researchers due to their ability to pleiotropically modulate a myriad of cellular mechanisms. A rapidly growing number of published studies have started to shed light on the mechanisms underlying the regulation of ROCK1 and ROCK2 via long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) in different types of cancer. Detailed analyses have suggested that lncRNAs may be characteristically divided into oncogenic and tumor suppressor lncRNAs. Several exciting recent discoveries have also indicated how different lncRNAs and circRNAs modulate ROCK1/2 and mediate multistep cancer onset and progression. The present review chronicles the major advances that have been made in our understanding of the regulatory role of ROCK1/2 in different types of cancer, and how wide-ranging lncRNAs and circRNAs potentiate ROCK-driven signaling by blocking the targeting activities of tumor suppressor microRNAs.

Correspondence to: Dr Ammad Ahmad Farooqi, Department of Molecular Oncology, Institute of Biomedical and Genetic Engineering, G9/1 Mauve Area, Islamabad 54000, Pakistan
E-mail: farooqiammadahmad@gmail.com

Key words: microRNA, cancer, signaling, apoptosis, rho-associated protein kinase

Contents

1. Introduction
2. Overview of the role of ROCKs in different types of cancer
3. Introduction of ncRNAs
4. Gaze through a 'molecular lens': ROCK-driven signaling
5. Negative regulation of ROCK1 by lncRNAs
6. Regulation of ROCK2 by lncRNAs: Oncogenic role
7. Tumor suppressor lncRNAs
8. circRNA-mediated regulation of ROCK1: Cancer-promoting roles of circRNAs and ROCK1
9. circRNAs and ROCK2 as tumor suppressors
10. Cancer-promoting roles of circRNAs and ROCK2
11. Concluding remarks

1. Introduction

Landmark discoveries in molecular oncology have started to shed light both on the underlying causes of cancer onset and progression, and on unmet clinical needs that have hastened re-interpretation of the recently emerging landscape of deregulated signaling pathways. Overexpression of oncogenes, loss of tumor suppressors, intra- and inter-tumor heterogeneity, and drug resistance are among the most extensively studied mechanisms (1-8).

Groundbreaking discoveries from the past decades have paradigmatically shifted the conceptual understanding of non-coding (nc)RNAs from being merely 'junk' transcriptional products to being considered as multifunctional regulators that contextually modulate a number of cellular processes, including transcription, post-transcriptional processing, chromatin remodeling and the regulation of cell signaling cascades. Together with an expansion of knowledge regarding the transcriptome space, it has become evident that a wide variety of RNA transcripts contain different microRNA (miRNA/miR)-binding sites (9-11).

Rapidly accumulating scientific evidence has enabled a transition to be made from a purely phenomenological to a more detailed mechanistic understanding that all RNA transcripts that contain miRNA-binding sites are able to regulate and communicate with each other by specifically competing for shared miRNAs, and they thereby serve as competing endogenous RNAs (ceRNAs). The diversity and complexity of known ceRNA interactions have increased exponentially with the discovery of an ever-increasing number of oncogenic and tumor suppressor long ncRNAs (lncRNAs) (12-15) and circular RNAs (16-18).

Rho-associated protein kinase (ROCK) is a serine/threonine protein kinase that was identified as a RhoGTP-binding protein, having a molecular mass of ~160 kDa (19,20). To date, two isoforms encoded by two different genes of ROCK (ROCK1 and ROCK2) have been investigated. ROCK1 and ROCK2 have been shown to fulfill major roles in carcinogenesis. These two proteins share an overall sequence similarity in their kinase domains of 92%, and at the amino-acid level, a similarity of 65% (21). Myosin light chain (MLC) is an important downstream substrate of ROCK1 that is phosphorylated by ROCK1 at Ser-19.

The present review exclusively focuses on cancer-related roles of lncRNAs, circular RNAs and ROCK1/2. PubMed (https://pubmed.ncbi.nlm.nih.gov/) was independently searched using the keywords 'lncRNA', 'ROCK1' and 'ROCK2'. All the articles were carefully screened and shortlisted for inclusion in the manuscript. Only those articles were selected which provided the findings about ncRNAs and ROCK1/ROCK2 exclusively in cancers.

The present review aims to summarize the interplay between ncRNAs and ROCK proteins in different types of cancer. First, a mechanistic overview of the ROCK proteins, and their key role in carcinogenesis, is provided. Subsequently, the review features an exclusive focus on how ncRNAs, particularly lncRNAs and circular RNAs (circRNAs), have been shown to interact with ROCK1 and ROCK2 in a wide variety of different types of cancer.

2. Overview of the role of ROCKs in different types of cancer

Transcription factor AP2-γ (TFAP2C) has been shown to enhance chemoresistance in colorectal cancer cells by stimulating the expression of ROCK1 and ROCK2 (22). Treatment with 5-fluorouracil induced regression of tumors in mice inoculated subcutaneously with TFAP2C-silenced HCT116 cells. TFAP2C also transcriptionally upregulates ROCK1 and ROCK2 in colorectal cancer cells (Fig. 1). Administration of Y-27632, a ROCK1/ROCK2 inhibitor, caused a considerable decrease in chemoresistance and stemness in TFAP2C-overexpressing cells (22).

FERM domain-containing protein 5 (FRMD5) has been shown to serve as a tumor suppressor, markedly restricting the motility of cancer cells (23). FRMD5 also physically interacts with ROCK1 and inhibits its activity (Fig. 1). The FERM-associated domain of FRMD5 was shown to be critical for interaction with the N-terminal domain of ROCK1. FRMD5 knockdown induced an increase in the phosphorylated levels of MLC, whereas FRMD5 overexpression inhibited the phosphorylation of MLC (23). Collectively, these findings suggested that FRMD5 is able to structurally interact with ROCK1, interfering with the ROCK1-mediated phosphorylation of MLC. Therefore, FRMD5 may inhibit the migration of lung cancer cells through the inhibition of ROCK1 kinase activity.

6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) has been shown to play a critical role in the metastasis of osteosarcoma (24). In a previous study, ROCK2 downregulation led to an obvious decrease in the migratory and invasive abilities of 143B and U2-OS cells, whereas PFKFB3 upregulation rescued the ROCK2 knockdown-induced effects. Furthermore, ROCK2 inhibition caused a marked decrease in the proliferative capabilities of 143B and U2-OS cells, whereas PFKFB3 upregulation restored the proliferative abilities of the osteosarcoma cells. ROCK2 prevented ubiquitin-mediated degradation of PFKFB3; moreover, ROCK2 inhibition enhanced the process of PFKFB3 ubiquitination, whereas, conversely, overexpression of ROCK2 led to a decrease in the levels of ubiquitinated PFKFB3. ROCK2 inhibition reduced the levels of PFKFB3 in osteosarcoma cells, and finally, lung metastasis was not observed in mice inoculated with ROCK2-silenced osteosarcoma cells (24).

Matrix metalloproteinase 2 (MMP2) is another widely studied protein that is reportedly involved in the positive regulation of metastasis (25). ROCK2 was shown to both prevent degradation of MMP2 and to induce an increase in MMP2 levels in hepatic cellular carcinoma (HCC) cells (25).

Forkhead box M1 (FOXM1) has been shown to modulate ROCK-driven signaling (26). ROCK2 has been reported to be an important FOXM1D-binding protein (FOXM1D being a novel isoform of FOXM1). In a previous study, ROCK inhibitors (fasudil and Y-27632) induced actin depolymerization, markedly decrease the levels of phosphorylated MLC and altered the shape of FOXM1D-overexpressing colorectal cancer LoVo and SW-480 cells. FOXM1D-induced activation of ROCK also contributed to the destruction of cell junctions and enhanced cell motility. Downregulation of E-cadherin could also potentially be a contributory factor towards the destruction of cell junctions (26).

The RNA-binding protein Lin28A, which contains a CCHC-zinc finger RNA-binding domain and cold shock domain (27), has also been shown to physically interact with ROCK2 and promote metastasis. The growth rates of tumors in mice injected with ROCK2-silenced ovarian cancer cells were found to be markedly lowered. There was also a marked decrease in the number of metastatic nodules present on lung surfaces of the mice injected with ROCK2-silenced ovarian cancer cells (27).

3. Introduction of ncRNAs

Evidence from genome-wide analyses and preclinical studies, supported by recently identified molecular insights, has improved our understanding of the fundamental role of ncRNAs in different types of cancer. miRNAs are small (ranging from 18-24 nucleotides), single-stranded ncRNAs. Ever since their discovery in 1993, it has been generally understood that these small molecules fulfill important roles in gene regulation; their mechanism of action is based on their binding to the

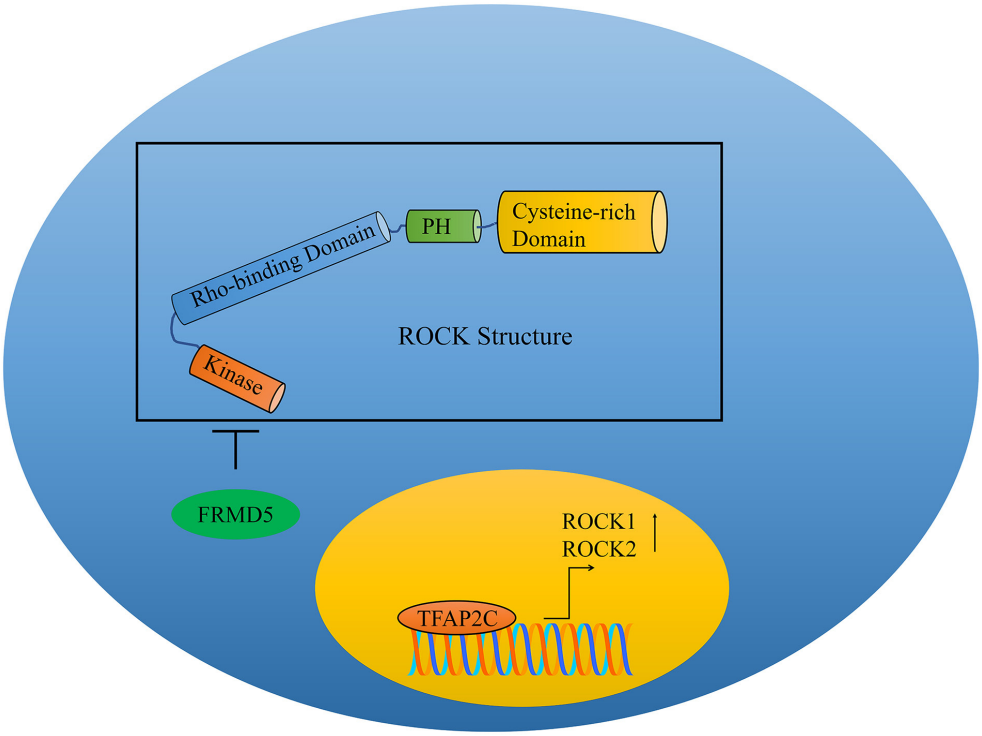

Figure 1. TFAP2C is a transcriptional factor and upregulates the expression of ROCK1 and ROCK2. ROCK is negatively regulated by FRMD5. PH, pleckstrin homology domain; TFAP2C, transcription factor AP2-γ; ROCK, rho-associated protein kinase; FRMD5, FERM domain-containing protein 5. Arrow indicates activation; $^{\perp}$ indicates inhibition.

3'-untranslated region of mRNA transcripts (28). lncRNAs also regulate gene transcription, although they consist of >200 nucleotides and are transcribed predominantly by RNA polymerase II. Similar to mRNAs, lncRNAs are also characterized by the presence of a 3'polyadenine tail (29). Regarding their role in cancer, both miRNAs and lncRNAs are considered to fulfill key roles in tumorigenesis and in tumor progression. Downregulation of certain tumor suppressor miRNAs is a common finding in breast, gynecological, prostate and lung cancer and brain tumors (30). The term 'oncomiR', which is used for several miRNAs, is indicative of the role of those miRNAs that have oncogenic functions. lncRNAs are also important in cancer, and a greatly expanding list of them has been noted to be correlated with particular types of cancer. A number of reviews have been recently published that provide a comprehensive overview of landmark discoveries in the field of ncRNAs (31-35).

4. Gaze through a 'molecular lens': ROCK-driven signaling

ROCK1-driven downstream signaling has been reported to occupy a central role in enhancing the invasive potential of cancer cells. The upcoming section focuses exclusively on lncRNAs and circRNAs reportedly involved in positive and negative regulation of ROCK in different cancer types.

Positive regulation of ROCK1 by lncRNAs: Tumorigenic role of lncRNAs

Cyclin-dependent kinase inhibitor 2B antisense RNA 1 (CDKN2B-AS1). CDKN2B-AS1 (also known as ANRIL) is an lncRNA that is frequently overexpressed in laryngeal squamous cell cancer (36). In a study by Liu *et al* (36) CDKN2B-AS1 knockdown was shown to cause the arrest cells in the G_1 phase and to decrease the number of cells in the S phase. Furthermore, the levels of proliferating cell nuclear antigen, an indicator of cell proliferation, were shown to be markedly decreased in cells where CDKN2B-AS1 had been knocked down. However, the levels of apoptosis-associated markers, in particular cleaved caspase-3 and cleaved poly (ADP-ribose) polymerase, were found to be markedly increased. Further experiments revealed that CDKN2B-AS1 knockdown induced apoptosis in AMC-HN-8 cells. Mechanistically, CDKN2B-AS1 regulated ROCK1 by blocking the activity of miRNA-324-5p in AMC-HN-8 cells. Taken together, the molecular analyses clearly suggested that miRNA-324-5p directly targeted ROCK1, whereas CDKN2B-AS1 sequestered miRNA-324-5p away from ROCK1, thereby relieving its inhibitory effects on ROCK1 (36).

Epidermal growth factor receptor-antisense RNA 1 (EGFR-AS1). EGFR-AS1 overexpression was shown to enhance the migratory and invasive capabilities of esophageal squamous cell carcinoma (ESCC) cells (37). miR-145 negatively regulated ROCK1 and decreased the invasive potential of ESCC cells. However, EGFR-AS1 sponged away miR-145 and promoted ROCK1 expression. Therefore, EGFR-AS1 was demonstrated to act as an oncogenic lncRNA that effectively potentiated ROCK1 expression (37).

Opa-interacting protein 5 antisense RNA 1 (OIP5-AS1). OIP5-AS1 is a cytoplasmic lncRNA (38). OIP5-AS1 inhibition was revealed to exert repressive effects on cell proliferation,

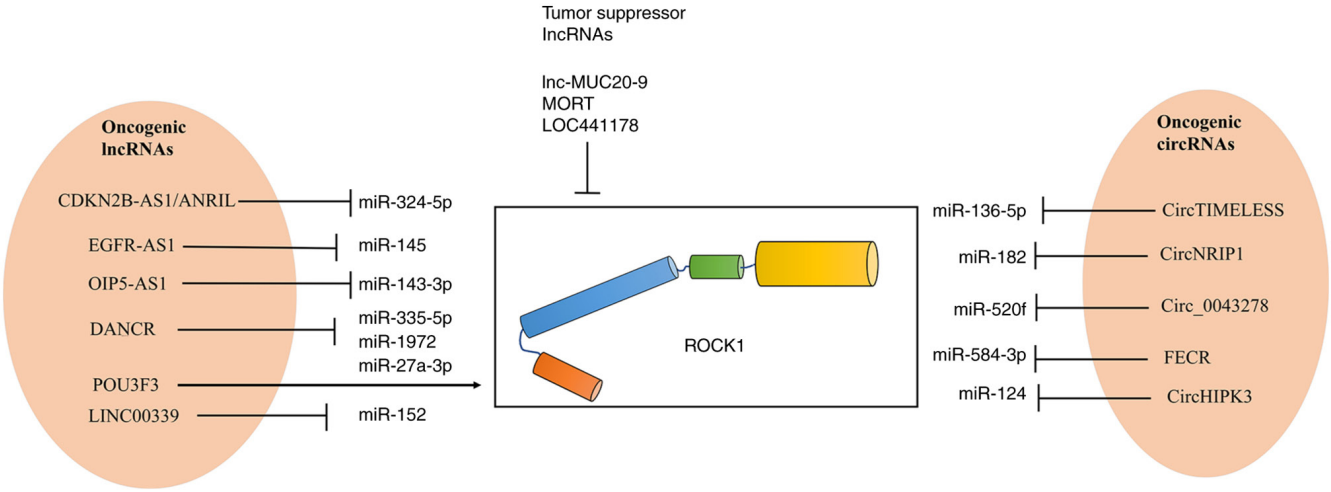

Figure 2. Oncogenic lncRNA- and circRNA-mediated inhibition of the ROCK1-targeting activity of tumor suppressor miRNAs. lncRNA, long non-coding RNA; circRNA, circular RNA; ROCK, rho-associated protein kinase; miR, microRNA; CDKN2B AS1, cyclin dependent kinase inhibitor 2B antisense RNA 1; EGFR AS1, epidermal growth factor receptor antisense RNA 1; OIP5 AS1, opa interacting protein 5 antisense RNA 1; DANCR, differentiation antagonizing non-protein coding RNA; POU3F3, POU domain class 3 transcription factor 2; NEAT1, nuclear paraspeckle assembly transcript 1; MORT, mortal obligate RNA transcript; FECR, FLI1 exonic circRNA. Arrow indicates activation; $^{\perp}$ indicates inhibition.

and OIP5-AS1 also acted as an inducer of apoptotic cell death in cervical cancer cells. Accordingly, ROCK1 was quantitatively controlled by miR-143-3p; however, OIP5-AS1 could interfere with the miR-143-3p-driven targeting of ROCK1 and potentiate its expression (38).

Differentiation antagonizing non-protein-coding RNA (DANCR). DANCR, a novel lncRNA, was found to be overexpressed in cervical cancer cells. Notably, DANCR stimulated the expression of ROCK1 mainly by interfering with the miR-335-5p-induced inhibition of ROCK1 (Fig. 2). Transfection of cervical cancer cells with miRNA-335-5p mimics or targeted inhibition of ROCK1 reversed the effects of upregulated DANCR (39).

Higher expression levels of DANCR were previously reported to be associated with a poor prognosis in clinical patients with osteosarcoma (40). miR-335-5p and miR-1972 both directly targeted ROCK1 mRNA expression. Transfection of cells with mimics of miR-1972 and miR-335-5p led to abrogation of DANCR-induced ROCK1 upregulation. DANCR overexpression also served a vital role in the metastasis of osteosarcoma cells to the lungs in xenografted mice (40).

In addition, DANCR was shown to stimulate both the proliferation and the metastasizing potential of HCC cells, whereas knockdown of DANCR exerted the opposite effects (41). Metastatic nodules on the surface of lungs were found to be considerably decreased in size in a xenograft mouse model established using DANCR-silenced cancer cells. Collectively, these experiments revealed that DANCR could act as a ceRNA, sequestering away miR-27a-3p to potentiate the expression of LIM domain kinase 1 (LIMK1) in HCC cells. DANCR activated the ROCK1/LIMK1/COFILIN1 signaling axis via inhibition of miR-27a-3p (41).

POU domain class 3 transcription factor 2 (POU3F3). POU3F3 acts as an oncogenic lncRNA, promoting an increase in the expression level of ROCK1 in prostate cancer cells (42). POU3F3 overexpression has also been shown to induce an increase in ROCK1 expression in prostate cancer cells (42).

Nuclear paraspeckle assembly transcript 1 (NEAT1). High-grade endometrioid and serous endometrial cancer are therapeutically resistant (43). NEAT1, an oncogenic lncRNA, was observed to be overexpressed in this cancer type; it acted as a molecular sponge and sequestered miR-361 away from STAT3. The orchestrated interaction of a myriad of oncogenic proteins was shown to induce drug resistance in endometrial cancer. The addition of miR-361 mimics significantly decreased paclitaxel resistance, whereas STAT3 overexpression enhanced paclitaxel resistance in SPAC-1-L and HI cells. Furthermore, NEAT1 inhibition resulted in decreases in the levels of ROCK1, STAT3, VEGF-A and WNT7A in SPAC-1-L cells (43).

LINC00339. LINC00339 has been shown to potentiate ROCK1 expression (44). miR-152 directly targeted ROCK1, although LINC00339 was shown to protect ROCK1 from being targeted by miR-152 in HCC cells. The metastatic spread of LINC00339-overexpressing HCC cells was also observed to be notably higher. However, LINC00339-silenced HCC cells did not metastasize to the lungs (44).

Small nucleolar RNA host gene 1 (SNHG1). SNHG1 was shown to interact with a tumor suppressor miRNA-101-3p, blocking its activity and potentiating ROCK1 expression (45). Levels of ROCK1, phosphorylated (p)-phosphoinositide 3-kinase (PI3K) and p-AKT were also found to be lowered in osteosarcoma cells transfected with miR-101-3p mimics; however, miR-101-3p knockdown by miR-101-3p inhibitor led to a robust increase in the levels of ROCK1, p-PI3K and p-AKT (45).

Taurine-upregulated gene 1 (TUG1). TUG1 has been shown to effectively sequester miR-145-5p away from ROCK1,

also stimulating ROCK1 expression (46). TUG1 suppression resulted in suppression of RhoA, ROCK1, MMP2 and MMP9 in laryngeal carcinoma cells (46).

E2F-mediated cell proliferation enhancing lncRNA (EPEL). EPEL has also been shown to promote ROCK1 expression (47). EPEL overexpression promoted both the migratory and invasive capabilities of osteosarcoma cells, and induced ROCK1 overexpression (47).

Terminal differentiation-induced non-coding RNA (TINCR). TINCR was also found to promote the migration and invasion of HCC cells (48). ROCK1 is a target of miR-214-5p. miR-214-5p targeted ROCK1 and markedly decreased the invasive potential of HCC cells; however, TINCR protected ROCK1 from miR-214-5p-mediated targeting (48).

LINC00452. LINC00452 was shown to promote ovarian carcinogenesis by antagonizing the miR-501-3p-mediated targeting of ROCK1. Additionally, LINC00452 physically interacted with ROCK1, thereby protecting it from ubiquitination. LINC00452 overexpression significantly promoted tumor growth in a xenograft model, although the simultaneous inhibition of ROCK1 markedly decreased the growth of the tumors in spite of the overexpression of LINC00452. Tumors developed from CaOV3 cells with overexpression of LINC00452, but where ROCK1 had been knocked down, were observed to be smaller in size (49).

LINC01087. LINC01087 has also been shown to act as an oncogenic lncRNA, as it effectively enhanced ROCK1 expression by blockade of miR-335-5p-mediated targeting of ROCK1 (50).

KCNMB2 antisense RNA 1 (KCNMB2-AS1). Ectopically expressed miR-374a-3p was shown to effect a significant reduction in the luciferase activity of ROCK1 in SK-MES-1 and H460 cells. However, KCNMB2-AS1 induced an increase in the expression of ROCK1 by sponging away miR-374a-3p. A marked decrease in the growth of the tumors developed from KCNMB2-AS1-silenced H460 cells was also observed. The level of KCNMB2-AS1 was notably decreased, whereas the expression of miR-374a-3p was elevated, in the tumor tissues of mice inoculated with KCNMB2-AS1-silenced H460 cells (51).

LINC00346. LINC00346 is capable of sponging miR-340-5p away from ROCK1 in glioma cells. In one study, tumor growth rates were found to be markedly decreased in mice subcutaneously injected with LINC00346-silenced U251 cells (52).

LINC00941. LINC00941 was found to enhance the metastatic potential of pancreatic cancer cells. miR-335-5p was shown to target ROCK1 and inhibit the metastatic spread. However, LINC00941 caused blockade of the miR-335-5p-mediated targeting of ROCK1. Tumors derived from LINC00941-silenced PANC-1 cells were also observed to be smaller in size. LINC00941 inhibition resulted in a marked decrease in the number of metastatic lesions on the surface of the liver and lungs of tumor-bearing mice (53).

5. Negative regulation of ROCK1 by lncRNAs

Lnc-MUC20-9 has been demonstrated to act as a tumor suppressor lncRNA, inhibiting the migratory potential of bladder cancer cells (54). Lnc-MUC20-9 has been reported to bind to ROCK1, thereby inhibiting its expression. Tumor growth was shown to be markedly decreased in mice transplanted with lnc-MUC20-9-overexpressing bladder cancer cells (54).

Mortal obligate RNA transcript (MORT) is a tumor suppressor lncRNA (55). Overexpression of MORT markedly decreased the proliferative ability of oral squamous cell carcinoma (OSCC) cells, and led to the downregulation of ROCK1. However, ROCK1 overexpression led to a significant increase in the proliferative ability of the OSCC cells. Furthermore, ROCK1 overexpression interfered with the inhibitory effects of MORT on the proliferation of OSCC cells (55).

LOC441178 also negatively regulated ROCK1 in OSCC cells (56). LOC441178 knockdown was shown to induce an increase in the ROCK1 levels (56).

6. Regulation of ROCK2 by lncRNAs: Oncogenic role

miR-4435-2HG, an oncogenic lncRNA, was shown to promote the expression of ROCK2, and inhibited the apoptotic death of ovarian cancer cells (57). miR4435-2HG overexpression induced the upregulation of ROCK2 in ovarian cancer UWB1.289 cells (57). Similarly, other lncRNAs that promote carcinogenesis have also been identified in ovarian cancer. TTN-AS1 blocked the ROCK2-targeting activity of miR-139-5p in SKOV3 cells, and the sizes and masses of subcutaneous tumors were observed to be significantly decreased in mice subcutaneously injected with TTN-AS1-silenced SKOV3 cells (58).

ZNFX1 antisense RNA 1 (ZFAS1) was also shown to promote the expression of ROCK2 by interfering with miR-3924-mediated targeting of ROCK2 in pancreatic cancer cells (59). Significant inhibition of liver metastasis was observed, although the extent of lung metastasis was not shown to be decreased in mice transplanted with ZFAS1-depleted SW1990 cells (59). These findings are of note, and future studies should concentrate on the identification of the underlying mechanisms.

EGFR-AS1 has also been found to be frequently overexpressed in bladder cancer cells (60). In bladder cancer HT-1197 cells, miR-381 directly targeted ROCK2 and decreased the invasive capability of the cells. miR-381-mediated targeting of ROCK2 was inhibited by EGFR-AS1 (60). LINC01638 overexpression induced ROCK2 upregulation in bladder cancer cells, although overexpression of ROCK2 did not exert a significant influence on LINC01638 expression (61). Overexpression of LINC01638 and ROCK2, however, led to an increase in both the migratory and invasive potentials of the bladder cancer cells. More importantly, ROCK2 inhibition abrogated the LINC01638-induced increase in the invasive potential of the cancer cells (61).

7. Tumor suppressor lncRNAs

HCC is a multifaceted disease, and lncRNAs have been shown to fulfill fundamental roles in the onset and progression of

cancer. Tumor suppressor lncRNAs have major roles with respect to inhibiting tumor invasion and spread. Overexpression of MAGI1 antisense RNA 3 (MAGI2-AS3) has been shown to induce the downregulation of ROCK2 (62). MAGI2-AS3 overexpression decreased the cell migratory and invasion rates, whereas ROCK2 abolished the effects of overexpression of MAGI2-AS3 (62). Taken together, these findings clearly suggested that the MAGI2-AS3-induced decrease in the cell migration and invasion rates was reversed by overexpression of ROCK2. Similarly, HAND2 antisense RNA 1 (HAND2-AS1) was found to induce downregulation of ROCK2 in HCC cells. HAND2-AS1 overexpression inhibited, whereas overexpression of ROCK2 potently enhanced, the migratory and invasive abilities of HCC cells (63).

8. circRNA-mediated regulation of ROCK1: Cancer-promoting roles of circRNAs and ROCK1

Recent advancements in circRNA-specific computational tools and high-throughput RNA sequencing have greatly helped in the development of state-of-the-art techniques for identification of circRNAs. circTIMELESS (hsa_circ_0000408) has been shown to serve as an oncogenic circRNA in lung squamous cell carcinoma (64). miR-136-5p negatively regulated ROCK1, although circTIMELESS antagonized the miR-136-5p-mediated targeting of ROCK1 and stimulated its expression (Fig. 2). Tumor growth was also markedly decreased in experimental mice injected with circTIMELESS-silenced cancer cells (64).

circNRIP1 has also been demonstrated to promote the expression of ROCK1, thereby enhancing carcinogenesis. The luciferase activity of ROCK1-expressing MGC-803 and AGS cells was significantly inhibited by overexpression of miR-182 (65). circNRIP1 sequestered miR-182 away from ROCK1, and promoted the expression of ROCK1 in gastric cancer cells. A marked decrease in Bcl-2 levels, with a concomitant increase in Bax levels, was also identified in circNRIP1-silenced gastric cancer cells (65).

circ_0043278 is another oncogenic circRNA that has been reported to be involved in enhancing the proliferative and migratory capabilities of non-small cell lung cancer (NSCLC) cells (66). miR-520f acted as a tumor suppressor and inhibited the growth and migration of NSCLC cells; however, miR-520f-mediated targeting of ROCK1 was impaired by circ_0043278 (66).

FLI1 exonic circRNA (FECR) has been shown to have critical roles in the migration and metastasis of cancer cells (67). FECR relieved the inhibitory effects of miR-584-3p on ROCK1 by sponging the miRNA away from its target. ROCK1 was found to be upregulated in miR-584-3p inhibitor-transfected SCLC cells. FECR silencing induced a decrease in the levels of ROCK1 in NCI-H446 and NCI-H1688 SCLC cells, whereas ectopic expression of FECR caused a marked upregulation of ROCK1 in NCI-H446 cells. Development of the tumors was observed to be markedly decreased in mice injected with FECR-silenced NCI-H446 cells. In addition, metastatic spread to the lungs and liver was significantly suppressed in mice inoculated with FECR-silenced NCI-H446 cells (67).

circHIPK3 has been shown to promote the proliferation of gallbladder cancer cells (68). Ectopic overexpression of circHIPK3 promoted the proliferative ability of gallbladder cancer cells, whereas an enforced expression of circHIPK3 exerted inhibitory effects on the levels of miR-124. ROCK1 was directly targeted by miR-124, although circHIPK3 was able to impair the tumor-suppressive effects exerted by miR-124 (68).

circ0001591 has also been shown to enhance the metastasizing potential of melanoma cells. Overexpression of circ0001591 caused an increase in the levels of PI3K and p-AKT. ROCK1 was directly targeted by miR-431-5p in melanoma cells. Furthermore, circ0001591 antagonized the miR-431-5p-mediated targeting of ROCK1 in melanoma cells (69). circ_101141 has also been shown to serve as an oncogenic circRNA, blocking miR-1297-mediated inhibition of ROCK1. The sizes of tumors were markedly decreased in mice injected with circ_101141-silenced Hep3B cells (70).

9. circRNAs and ROCK2 as tumor suppressors

Cisplatin-resistant gastric cancer cell lines have been demonstrated to have significantly lower ROCK2 and circCUL2 levels, and significantly higher miR-142-3p levels. In a previous study, circCUL2 acted as a tumor suppressor circRNA, sequestering away miR-142-3p. miR-142-3p was shown to act as an oncogenic miRNA, directly targeting ROCK2 and promoting carcinogenesis. Extensive tumor shrinkage was observed in mice injected with circCUL2-overexpressing SGC-7901 cells (71).

10. Cancer-promoting roles of circRNAs and ROCK2

circ_HN1 has been demonstrated to promote cancer progression by blocking the targeting of ROCK2 by miR-302b-3p. Tumors derived from circ_HN1-silenced HGC-27 cells were found to be smaller in size. The levels of circ_HN1 and ROCK2 were also shown to be decreased in the tumor tissues of xenograft model mice established with circ_HN1-silenced HGC-27 cells (72).

11. Concluding remarks

The present review has critically discussed recent information associated with the regulation of ROCK1/2 by lncRNAs and circRNAs, providing an updated translational perspective that may be useful in terms of guiding the selection of optimal targets and disease-tailored interventions. ROCK-driven signaling has been shown to serve as a linchpin during various steps of cancer. Pharmacological and pharmaceutical researchers have started to focus their attention on the design and development of chemical inhibitors for ROCK1 and ROCK2. Nevertheless, much further research needs to be done, since sufficient experimental evidence associated with epigenetic regulation of ROCK1/2 in different types of cancer remains lacking. However, a series of cutting-edge studies have started to shed light on the post-transcriptional regulation of ROCK1/2 by lncRNAs and circRNAs in different types of cancer. These aspects are of note and allow researchers to analyze various lncRNAs that are involved in the positive regulation of ROCK1/2 in different cancer types. lncRNAs regulate the expression of ROCK1/2 by interfering with the miRNA-mediated targeting of ROCK, and this knowledge has already been carried forward to tests conducted in preclinical

models. Therefore, there is a need to pursue the interplay between ncRNAs and ROCK1/2 more comprehensively in order for scientists to critically evaluate the efficacy of ROCK inhibitors in different types of cancer.

Acknowledgements

Not applicable.

Funding

This study was financed through a statutory subsidy by the Minister of Science and Higher Education as a part of the research grant SUB.C280.21.023 (record number in the Simple System) and statutory resources of Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Scienses (2021/06).

Availability of data and materials

Not applicable.

Authors' contributions

RZ and HN collected raw data after extensive browsing through Scopus and PubMed. JS, RM and EP shortlisted the most relevant and English language based research articles for inclusion in this review. AAF, RA, MG and RB wrote the manuscript. RZ and HN constructed the figures. JS, RM and EP carefully edited the manuscript for technical errors and accurate scientific presentation. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they do not have any competing interests.

References

1. Fares J, Fares MY, Khachfe HH, Salhab HA and Fares Y: Molecular principles of metastasis: A hallmark of cancer revisited. Signal Transduct Target Ther 5: 28, 2020.
2. Weber J, Braun CJ, Saur D and Rad R: In vivo functional screening for systems-level integrative cancer genomics. Nat Rev Cancer 20: 573-593, 2020.
3. Lin A and Sheltzer JM: Discovering and validating cancer genetic dependencies: Approaches and pitfalls. Nat Rev Genet 21: 671-682, 2020.
4. Allam M, Cai S and Coskun AF: Multiplex bioimaging of single-cell spatial profiles for precision cancer diagnostics and therapeutics. NPJ Precis Oncol 4: 11, 2020.
5. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr and Kinzler KW: Cancer genome landscapes. Science 339: 1546-1558, 2013.
6. Noble ME, Endicott JA and Johnson LN: Protein kinase inhibitors: Insights into drug design from structure. Science 303: 1800-1805, 2004.
7. Reubold TF and Eschenburg S: A molecular view on signal transduction by the apoptosome. Cell Signal 24: 1420-1425, 2012.
8. Janse van Rensburg HJ and Yang X: The roles of the Hippo pathway in cancer metastasis. Cell Signal 28: 1761-1772, 2016.
9. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, *et al*: A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 103: 2257-2261, 2006.
10. Lytle JR, Yario TA and Steitz JA: Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5'UTR as in the 3'UTR. Proc Natl Acad Sci USA 104: 9667-9672, 2007.
11. Khraiwesh B, Arif MA, Seumel GI, Ossowski S, Weigel D, Reski R and Frank W: Transcriptional control of gene expression by microRNAs. Cell 140: 111-122, 2010.
12. Cabili MN, Dunagin MC, McClanahan PD, Biaesch A, Padovan-Merhar O, Regev A, Rinn JL and Raj A: Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution. Genome Biol 16: 20, 2015.
13. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, Barrette TR, Prensner JR, Evans JR, Zhao S, *et al*: The landscape of long noncoding RNAs in the human transcriptome. Nat Genet 47: 199-208, 2015.
14. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, *et al*: Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458: 223-227, 2009.
15. Chiu HS, Somvanshi S, Patel E, Chen TW, Singh VP, Zorman B, Patil SL, Pan Y, Chatterjee SS; Cancer Genome Atlas Research Network, *et al*: Pan-cancer analysis of lncRNA regulation supports their targeting of cancer genes in each tumor context. Cell Rep 23: 297-312.e12, 2018.
16. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, *et al*: Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495: 333-338, 2013.
17. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK and Kjems J: Natural RNA circles function as efficient microRNA sponges. Nature 495: 384-388, 2013.
18. Salzman J, Gawad C, Wang PL, Lacayo N and Brown PO: Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. PLoS One 7: e30733, 2012.
19. Shibata H, Oishi K, Yamagiwa A, Matsumoto M, Mukai H and Ono Y: PKNbeta interacts with the SH3 domains of Graf and a novel Graf related protein, Graf2, which are GTPase activating proteins for Rho family. J Biochem 130: 23-31, 2001.
20. Ishizaki T, Maekawa M, Fujisawa K, Okawa K, Iwamatsu A, Fujita A, Watanabe N, Saito Y, Kakizuka A, Morii N and Narumiya S: The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase. EMBO J 15: 1885-1893, 1996.
21. Leung T, Manser E, Tan L and Lim L: A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. J Biol Chem 270: 29051-29054, 1995.
22. Wang X, Sun D, Tai J, Chen S, Yu M, Ren D and Wang L: TFAP2C promotes stemness and chemotherapeutic resistance in colorectal cancer via inactivating hippo signaling pathway. J Exp Clin Cancer Res 37: 27, 2018.
23. Hu J, Niu M, Li X, Lu D, Cui J, Xu W, Li G, Zhan J and Zhang H: FERM domain-containing protein FRMD5 regulates cell motility via binding to integrin β5 subunit and ROCK1. FEBS Lett 588: 4348-4356, 2014.
24. Deng X, Yi X, Deng J, Zou Y, Wang S, Shan W, Liu P, Zhang Z, Chen L and Hao L: ROCK2 promotes osteosarcoma growth and metastasis by modifying PFKFB3 ubiquitination and degradation. Exp Cell Res 385: 111689, 2019.
25. Huang D, Du X, Yuan R, Chen L, Liu T, Wen C, Huang M, Li M, Hao L and Shao J: Rock2 promotes the invasion and metastasis of hepatocellular carcinoma by modifying MMP2 ubiquitination and degradation. Biochem Biophys Res Commun 453: 49-56, 2014.
26. Zhang X, Zhang L, Du Y, Zheng H, Zhang P, Sun Y, Wang Y, Chen J, Ding P, Wang N, *et al*: A novel FOXM1 isoform, FOXM1D, promotes epithelial-mesenchymal transition and metastasis through ROCKs activation in colorectal cancer. Oncogene 36: 807-819, 2017.

27. Zhong Y, Yang S, Wang W, Wei P, He S, Ma H, Yang J, Wang Q, Cao L, Xiong W, *et al*: The interaction of Lin28A/Rho associated coiled-coil containing protein kinase2 accelerates the malignancy of ovarian cancer. Oncogene 38: 1381-1397, 2019.
28. Hsu PW, Huang HD, Hsu SD, Lin LZ, Tsou AP, Tseng CP, Stadler PF, Washietl S and Hofacker IL: miRNAMap: Genomic maps of microRNA genes and their target genes in mammalian genomes. Nucleic Acids Res 34 (Database Issue): D135-D139, 2006.
29. Du Z, Sun T, Hacisuleyman E, Fei T, Wang X, Brown M, Rinn JL, Lee MG, Chen Y, Kantoff PW and Liu XS: Integrative analyses reveal a long noncoding RNA-mediated sponge regulatory network in prostate cancer. Nat Commun 7: 10982, 2016.
30. Kumar MS, Lu J, Mercer KL, Golub TR and Jacks T: Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 39: 673-677, 2007.
31. Treiber T, Treiber N and Meister G: Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nat Rev Mol Cell Biol 20: 5-20, 2019.
32. Nair L, Chung H and Basu U: Regulation of long non-coding RNAs and genome dynamics by the RNA surveillance machinery. Nat Rev Mol Cell Biol 21: 123-136, 2020.
33. Geisler S and Coller J: RNA in unexpected places: Long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol 14: 699-712, 2013.
34. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB and Kjems J: The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet 20: 675-691, 2019.
35. Santer L, Bär C and Thum T: Circular RNAs: A novel class of functional RNA molecules with a therapeutic perspective. Mol Ther 27: 1350-1363, 2019.
36. Liu F, Xiao Y, Ma L and Wang J: Regulating of cell cycle progression by the lncRNA CDKN2B-AS1/miR-324-5p/ROCK1 axis in laryngeal squamous cell cancer. Int J Biol Markers 35: 47-56, 2020.
37. Feng Z, Li X, Qiu M, Luo R, Lin J and Liu B: LncRNA EGFR-AS1 Upregulates ROCK1 by sponging miR-145 to promote esophageal squamous cell carcinoma cell invasion and migration. Cancer Biother Radiopharm 35: 66-71, 2020.
38. Song L, Wang L, Pan X and Yang C: lncRNA OIP5-AS1 targets ROCK1 to promote cell proliferation and inhibit cell apoptosis through a mechanism involving miR-143-3p in cervical cancer. Braz J Med Biol Res 53: e8883, 2020.
39. Liang H, Zhang C, Guan H, Liu J and Cui Y: LncRNA DANCR promotes cervical cancer progression by upregulating ROCK1 via sponging miR-335-5p. J Cell Physiol 234: 7266-7278, 2019.
40. Wang Y, Zeng X, Wang N, Zhao W, Zhang X, Teng S, Zhang Y and Lu Z: Long noncoding RNA DANCR, working as a competitive endogenous RNA, promotes ROCK1-mediated proliferation and metastasis via decoying of miR-335-5p and miR-1972 in osteosarcoma. Mol Cancer 17: 89, 2018.
41. Guo D, Li Y, Chen Y, Zhang D, Wang X, Lu G, Ren M, Lu X and He S: DANCR promotes HCC progression and regulates EMT by sponging miR-27a-3p via ROCK1/LIMK1/COFILIN1 pathway. Cell Prolif 52: e12628, 2019.
42. Wan X, Xiang J, Zhang Q and Bian C: Long noncoding RNA POU3F3 promotes cancer cell proliferation in prostate carcinoma by upregulating rho-associated protein kinase 1. J Cell Biochem: Nov 26, 2018 (Epub ahead of print). doi: 10.1002/jcb.28101.
43. Dong P, Xiong Y, Yue J, Xu D, Ihira K, Konno Y, Kobayashi N, Todo Y and Watari H: Long noncoding RNA NEAT1 drives aggressive endometrial cancer progression via miR-361-regulated networks involving STAT3 and tumor microenvironment-related genes. J Exp Clin Cancer Res 38: 295, 2019.
44. Chen K and Zhang L: LINC00339 regulates ROCK1 by miR-152 to promote cell proliferation and migration in hepatocellular carcinoma. J Cell Biochem 120: 14431-14443, 2019.
45. Deng R, Zhang J and Chen J: lncRNA SNHG1 negatively regulates miRNA-101-3p to enhance the expression of ROCK1 and promote cell proliferation, migration and invasion in osteosarcoma. Int J Mol Med 43: 1157-1166, 2019.
46. Zhuang S, Liu F and Wu P: Upregulation of long noncoding RNA TUG1 contributes to the development of laryngocarcinoma by targeting miR-145-5p/ROCK1 axis. J Cell Biochem 120: 13392-13402, 2019.
47. Chen S, Liu Z, Lu S and Hu B: EPEL promotes the migration and invasion of osteosarcoma cells by upregulating ROCK1. Oncol Lett 17: 3133-3140, 2019.
48. Hu M, Han Y, Zhang Y, Zhou Y and Ye L: lncRNA TINCR sponges miR-214-5p to upregulate ROCK1 in hepatocellular carcinoma. BMC Med Genet 21: 2, 2020.
49. Yang J, Wang WG and Zhang KQ: LINC00452 promotes ovarian carcinogenesis through increasing ROCK1 by sponging miR-501-3p and suppressing ubiquitin-mediated degradation. Aging (Albany NY) 12: 21129-21146, 2020.
50. She JK, Fu DN, Zhen D, Gong GH and Zhang B: LINC01087 is highly expressed in breast cancer and regulates the malignant behavior of cancer cells through miR-335-5p/Rock1. Onco Targets Ther 13: 9771-9783, 2020.
51. Yang H, Wang Z and Wang Z: Long noncoding RNA KCNMB2-AS1 increases ROCK1 expression by sponging microRNA-374a-3p to facilitate the progression of non-small-cell lung cancer. Cancer Manag Res 12: 12679-12695, 2020.
52. Chen X, Li D, Chen L, Hao B, Gao Y, Li L, Zhou C, He X and Cao Y: Long noncoding RNA LINC00346 promotes glioma cell migration, invasion and proliferation by up-regulating ROCK1. J Cell Mol Med 24: 13010-13019, 2020.
53. Wang J, He Z, Xu J, Chen P and Jiang J: Long noncoding RNA LINC00941 promotes pancreatic cancer progression by competitively binding miR-335-5p to regulate ROCK1-mediated LIMK1/Cofilin-1 signaling. Cell Death Dis 12: 36, 2021.
54. Dai R, Zhou Y, Chen Z, Zou Z, Pan Z, Liu P and Gao X: Lnc-MUC20-9 binds to ROCK1 and functions as a tumor suppressor in bladder cancer. J Cell Biochem 121: 4214-4225, 2020.
55. Jin Z, Jiang S, Jian S and Shang Z: Long noncoding RNA MORT overexpression inhibits cancer cell proliferation in oral squamous cell carcinoma by downregulating ROCK1. J Cell Biochem: Feb 25, 2019 (Epub ahead of print). doi: 10.1002/jcb.28449.
56. Xu K, Tian H, Zhao S, Yuan D, Jiang L, Liu X, Zou B and Zhang J: Long Noncoding RNA LOC441178 reduces the invasion and migration of squamous carcinoma cells by targeting ROCK1. Biomed Res Int 2018: 4357647, 2018.
57. Hu J, Wang L, Zhao W, Huang Y, Wang Z and Shen H: mi-R4435-2HG promotes proliferation and inhibits apoptosis of cancer cells in ovarian carcinoma by upregulating ROCK2. Oncol Lett 19: 1305-1309, 2020.
58. Liu X, Li Y, Wen J, Qi T and Wang Y: Long non-coding RNA TTN-AS1 promotes tumorigenesis of ovarian cancer through modulating the miR-139-5p/ROCK2 axis. Biomed Pharmacother 125: 109882, 2020.
59. Liu J, Zhu Y and Ge C: LncRNA ZFAS1 promotes pancreatic adenocarcinoma metastasis via the RHOA/ROCK2 pathway by sponging miR-3924. Cancer Cell Int 20: 249, 2020.
60. Yuan S, Luan X, Chen H, Shi X and Zhang X: Long non-coding RNA EGFR-AS1 sponges micorRNA-381 to upregulate ROCK2 in bladder cancer. Oncol Lett 19: 1899-1905, 2020.
61. Yuan S, Luan X, Han G, Guo K, Wang S and Zhang X: LINC01638 lncRNA mediates the postoperative distant recurrence of bladder cancer by upregulating ROCK2. Oncol Lett 18: 5392-5398, 2019.
62. Fang G, Wang J, Sun X, Xu R, Zhao X, Shao L, Sun C and Wang Y: LncRNA MAGI2-AS3 is downregulated in the distant recurrence of hepatocellular carcinoma after surgical resection and affects migration and invasion via ROCK2. Ann Hepatol 19: 535-540, 2020.
63. Jiang L, He Y, Shen G, Ni J, Xia Z, Liu H, Cao Y and Li X: lncRNA HAND2-AS1 mediates the downregulation of ROCK2 in hepatocellular carcinoma and inhibits cancer cell proliferation, migration and invasion. Mol Med Rep 21: 1304-1309, 2020.
64. Zhang W, Shi J, Cheng C and Wang H: CircTIMELESS regulates the proliferation and invasion of lung squamous cell carcinoma cells via the miR-136-5p/ROCK1 axis. J Cell Physiol 235: 5962-5971, 2020.
65. Liang L and Li L: Down-regulation of circNRIP1 promotes the apoptosis and inhibits the migration and invasion of gastric cancer cells by miR-182/ROCK1 Axis. Onco Targets Ther 13: 6279-6288, 2020.
66. Cui J, Li W, Liu G, Chen X, Gao X, Lu H and Lin D: A novel circular RNA, hsa_circ_0043278, acts as a potential biomarker and promotes non-small cell lung cancer cell proliferation and migration by regulating miR-520f. Artif Cells Nanomed Biotechnol 47: 810-821, 2019.
67. Li L, Li W, Chen N, Zhao H, Xu G, Zhao Y, Pan X, Zhang X, Zhou L, Yu D, *et al*: FLI1 Exonic circular RNAs as a novel oncogenic driver to promote tumor metastasis in small cell lung cancer. Clin Cancer Res 25: 1302-1317, 2019.
68. Kai D, Yannian L, Yitian C, Dinghao G, Xin Z and Wu J: Circular RNA HIPK3 promotes gallbladder cancer cell growth by sponging microRNA-124. Biochem Biophys Res Commun 503: 863-869, 2018.

69. Yin D, Wei G, Yang F and Sun X: Circular RNA has circ 0001591 promoted cell proliferation and metastasis of human melanoma via ROCK1/PI3K/AKT by targeting miR-431-5p. Hum Exp Toxicol 40: 310-324, 2021.
70. Zhang T, Zhang L, Han D, Tursun K and Lu X: Circular RNA hsa_Circ_101141 as a competing endogenous RNA facilitates Tumorigenesis of hepatocellular carcinoma by regulating miR-1297/ROCK1 pathway. Cell Transplant 29: 963689720948016, 2020.
71. Peng L, Sang H, Wei S, Li Y, Jin D, Zhu X, Li X, Dang Y and Zhang G: circCUL2 regulates gastric cancer malignant transformation and cisplatin resistance by modulating autophagy activation via miR-142-3p/ROCK2. Mol Cancer 19: 156, 2020.
72. Wang D, Jiang X, Liu Y, Cao G, Zhang X and Kuang Y: Circular RNA circ_HN1 facilitates gastric cancer progression through modulation of the miR-302b-3p/ROCK2 axis. Mol Cell Biochem 476: 199-212, 2021.