BBA - Reviews on Cancer 1881 (2026) 189508

Contents lists available at ScienceDirect

BBA - Reviews on Cancer

journal homepage: www.elsevier.com/locate/bbacan

Review Article

Role of microRNAs in the regulation of RKIP and signaling pathways in cancer☆

Graziana Spoto [a], Massimo Libra [a,b,*], Luca Falzone [a,*]

[a] Department of Biomedical and Biotechnological Sciences, University of Catania, 95123 Catania, Italy

[b] Research Center for Prevention, Diagnosis and Treatment of Cancer, University of Catania, 95123 Catania, Italy

ARTICLE INFO

Keywords:
RKIP
PEBP1
microRNAs
Long non-coding RNA
Oncology
Tumor suppressor

ABSTRACT

Raf kinase inhibitor protein (RKIP), also known as Phosphatidyl Ethanolamine Binding Protein (PEBP1), is a pivotal modulator of multiple intracellular signaling cascades involved in tumorigenesis, progression, metastasis, and cancer therapy resistance. In recent years, increasing evidence has highlighted the regulatory role of non-coding RNAs, particularly microRNAs (miRNAs), in modulating RKIP expression and activity across various types of cancer. This review aims to comprehensively summarize current knowledge on the post-transcriptional regulation of RKIP by miRNAs, elucidating their impact on tumor biology.

For this purpose, a systematic analysis of published experimental studies was conducted, focusing on both solid and hematological malignancies. The review discusses how miRNAs, such as miR-23a, miR-27a, miR-224, miR-181a, and others, directly or indirectly suppress RKIP, contributing to enhanced proliferation, invasion, epithelial-mesenchymal transition (EMT), cancer stem cell (CSC) traits, and radioresistance. Additionally, long non-coding RNAs (lncRNAs) like XIST and PEBP1P2 were identified as factors able to modulate RKIP suppression by acting as molecular sponges for miRNAs or stabilizing RKIP transcripts.

All the data presented in the manuscript are supported by diverse experimental approaches, including transcriptional analyses, functional in vitro assays (migration, invasion, apoptosis), gain- and loss-of-function experiments, luciferase reporter assays, and in vivo xenograft models, further validating the miRNA-RKIP axis involved in the progression of multiple tumors.

In conclusion, this review provides an integrated view of the complex post-transcriptional network governing RKIP regulation in cancer, underscoring the potential of targeting RKIP-associated non-coding RNA axes for innovative therapeutic strategies aimed at halting tumor progression and overcoming treatment resistance.

1. Introduction

Cancer development and progression are driven not only by oncogene activation but also by the loss of tumor suppressor gene function, which disrupts key regulatory processes such as proliferation, apoptosis, migration, and differentiation. The imbalance of intracellular signaling facilitates malignant transformation, tumor progression, and therapeutic resistance [1,2].

Among the tumor suppressor genes, Raf Kinase Inhibitory Protein (RKIP), also known as phosphatidylethanolamine-binding protein 1 (PEBP1), has gained significant attention [3]. Notably, RKIP negatively regulates key signaling pathways involved in tumor cell proliferation, invasion, and metastasis, including the mitogen-activated protein kinase (MAPK) [4], G protein-coupled receptors (GPCRs) [5], nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [6], Signal Transducer and Activator of Transcription 3 (STAT3) [7,8], and Glycogen Synthase Kinase-3 (GSK-3) pathways [9]. The regulation of all these pathways has suggested the pivotal role of RKIP in both cancer development and progression in a wide spectrum of human cancers, highlighting the need for more in-depth studies on the mechanisms behind the dysregulation of this binding protein.

From a functional point of view, RKIP downregulation is frequently associated with high-grade tumor stages, metastatic and therapeutic-resistant phenotypes. Thus, it represents a negative marker predictive of poor clinical outcome [10–12]. Several studies have reported the role of RKIP as a metastasis suppressor, highlighting its involvement in

☆ This article is part of a Special issue entitled: 'Targeting YY1 and RKIP' published in BBA - Reviews on Cancer.

* Corresponding authors at: Department of Biomedical and Biotechnological Sciences, University of Catania, 95123 Catania, Italy.
E-mail addresses: m.libra@unict.it (M. Libra), luca.falzone@unict.it (L. Falzone).

https://doi.org/10.1016/j.bbcan.2025.189508
Received 30 June 2025; Received in revised form 30 August 2025; Accepted 3 December 2025
Available online 9 December 2025

various steps of the metastatic process, including cell migration, invasion and the epithelial-mesenchymal transition (EMT) mechanism [13–17].

The mechanisms underlying RKIP suppression in tumors are multifactorial and involve genetic and, more predominantly, epigenetic modifications [18]. Unlike many tumor suppressors that are frequently mutated or deleted, RKIP is rarely genetically altered in human cancers [19,20]. Instead, its downregulation is commonly the result of regulatory events at the transcriptional [21,22], post-transcriptional [23,24], and post-translational levels [25,26], reflecting the dynamic plasticity of cancer cell signaling.

Among epigenetic mechanisms, microRNAs (miRNAs) have garnered particular attention for their pivotal role in post-transcriptional gene silencing. Therefore, this review wants to focus the discussion on the miRNA-mediated regulation of RKIP that may be responsible for the RKIP downregulation observed in multiple cancers. Notably, miRNAs are small, non-coding RNAs that bind to complementary sequences in the 3′ untranslated region (3′UTR) of target mRNAs, leading to mRNA degradation or translational repression [27,28]. Depending on their targets, miRNAs can act either as oncomiRs, promoting oncogenesis by repressing tumor suppressor genes, or as tumor-suppressor miRNAs, by downregulating oncogenes [29]. As will be widely discussed in the next sections, several oncomiRs have been shown to directly target RKIP, thereby diminishing its tumor-suppressive functions and facilitating malignant transformation and progression [30]. Concomitantly, miRNAs may also regulate upstream and downstream RKIP effectors, thus further modulating the complex interactive network regulated by RKIP. Therefore, understanding the regulation of RKIP by miRNAs offers valuable insights not only from a mechanistic standpoint but also from a translational perspective to reveal novel biomarkers for cancer diagnosis and prognosis, as well as to identify promising therapeutic targets for restoring tumor suppressor activity in RKIP-deficient cancers.

Overall, the purpose of this review is to provide an updated overview of the current knowledge regarding RKIP regulation in cancer, with a particular emphasis on epigenetic mechanisms mediated by miRNAs. We will summarize key signaling pathways modulated by RKIP, explore how its expression is regulated across multiple tumor types, and highlight the functional impact of miRNA–RKIP interactions in breast, prostate, lung, liver, gastric, and other cancers. Ultimately, this review aims to deepen our understanding of RKIP's role in cancer biology and to shed light on the diagnostic, prognostic, and therapeutic relevance of its regulatory network.

2. RKIP in cancer

2.1. Key signaling pathway regulated by RKIP

RKIP is a dynamic protein existing in multiple conformational states [31]. The plasticity of its structure, primarily due to its conserved ligand-binding pocket and the allosteric structure, responds to different functional roles across multiple signaling networks [32].

In physiological conditions, RKIP modulates various signaling cascades primarily related to cell integrity, cell growth, apoptosis, survival, and differentiation [33,34].

Due to the centrality of RKIP-regulated signaling pathways involved in the maintenance of integrity and balance of biological systems, its depletion results in the alteration of normal cellular homeostasis, leading to numerous diseases, including cancer [33,35]. Numerous studies demonstrated that RKIP inactivation leads to tumor progression and metastasis initiation as demonstrated in cancer cells, mainly due to the dysregulation of MAPKs, GPCRs, NF-kB, STAT3 and GSK3β pathways (Fig. 1) [13,34,36].

2.1.1. RKIP regulation of the MAP kinase signaling pathway

The MAPK signal transduction pathway plays a pivotal role in the regulation of cell proliferation, differentiation, apoptosis, and stress response [37].

Notably, the Raf-1/MEK/ERK pathway induces proliferative signals or stress stimuli from the membrane to the nucleus through a multistep process involving a cascade of protein kinases [38]. Specifically, the activation of the tyrosine kinase receptors (RTKs) by extracellular stimuli leads to the initiation of the signal transduction cascade responsible for the consequent activation of Ras, which recruits and activates Raf-1. The activation of Raf-1 determines the phosphorylation of MEK1/2 and subsequently of ERK1/2. Activated ERKs translocate to the nucleus, where they regulate gene expression by the transactivation of transcription factors that promote cell growth and differentiation [39–43]. In cancer, the hyperactivation of the MAPK pathway notably leads to the promotion, development and progression of tumor cells [44,45].

In this context, RKIP was first recognized as an inhibitor of the MAPK signaling by binding Raf-1 [4]. Its interaction with the N-region of the Raf-1 kinase domain, at residues 93–134, prevents Raf-1 phosphorylation at Ser338 and Tyr340/341 by PAK and Src Kinase with the consequent inactivation of the entire MAPK cascade [4,46–48]. It was observed that RKIP can also form ternary complexes with MEK and, to a lesser extent, ERK. Since MEK and Raf-1 bind RKIP on the same binding

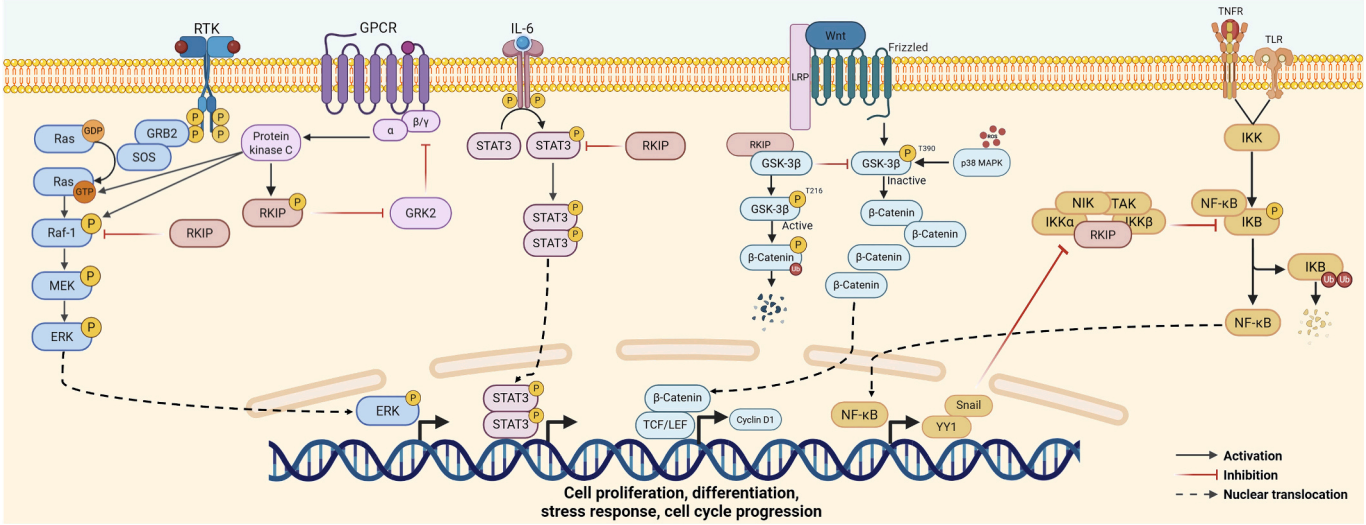

Fig. 1. Molecular pathways regulated by RKIP.

site, their interaction with RKIP is mutually exclusive [46]. In contrast, MEK and ERK can simultaneously interact with RKIP. Therefore, RKIP not only inhibits the MAPK pathway by binding Raf-1, but also acts as a competitive inhibitor of MEK phosphorylation, dissociating the Raf-1/MEK complex [46].

The overexpression of RKIP interferes with the activation of the MAPK signaling cascade and the inhibition of downstream AP-1 transcription factor [4].

Furthermore, RKIP depletion does not affect B-Raf phosphorylation, suggesting that RKIP specifically suppresses Raf-1 protein [47]. Thus, its downregulation activates the MAPK cascade through a Raf-1-dependent mechanism, promoting cancer development and progression.

2.1.2. RKIP regulation of the NF-kB pathway

NF-kB is a transcription factor activated in response to environmental changes such as inflammation, infection, and stress conditions, exerting pro-survival and anti-apoptotic functions. Dysregulation of the NF-kB pathway is associated with tumorigenesis, EMT, metastasis and drug resistance [49–51]. In its inactive form, NF-kB is retained in the cytoplasm bound to the inhibitory kappa B (IkB). In response to different stimuli, the IkappaB kinase (IKK) mediates the phosphorylation and degradation of IkB. This process releases active NF-kB, which translocates to the nucleus and promotes the transcription of several genes involved in cell migration and invasion (matrix metalloproteinases), in cell adhesion (selectins, integrins), and EMT modulation (Snail, TWIST) [52–56]. NF-kB has also been reported as a regulator of YY1, a transcription factor implicated in EMT and metastasis formation [57]. Furthermore, it has been demonstrated that the transcription of Snail can be directly regulated by both NF-kB and YY1 [58]. In cancer cells, the functional activation of NF-kB along with YY1 overexpression leads to increased Snail overexpression, which in turn suppresses RKIP [59]. In this intricate NF-kB/YY1/Snail circuit, RKIP functions as a negative regulator, disrupting the pro-metastatic loop by inhibiting NF-kB signaling with the simultaneous inactivation of its downstream effectors YY1 and Snail [60].

The inactivation of NF-kB mediated by RKIP is related to the inhibition of upstream kinases, including transforming growth factor beta-activated kinase 1 (TAK1), NF-kappaB-inducing kinase (NIK), IKKα and IKKβ.

Specifically, it has been observed that RKIP suppresses the TNFα- and IL-1β-mediated activation of the NF-kB pathway, resulting in the reversal of tumor chemoresistance and increased sensitivity to apoptosis [6]. Notably, YY1 acts as a transcriptional repressor of the death receptor DR5, which is crucial for TRAIL-induced apoptosis [61]. By negatively regulating YY1, RKIP overexpression enhances DR5 expression that results in an increased TRAIL-mediated apoptotic response [62]. Conversely, RKIP-deficient cells exhibit resistance to TRAIL-induced apoptosis and chemotherapeutic agents [63]. Moreover, in cancer cells, RKIP overexpression downregulates the anti-apoptotic proteins Bcl-xL and XIAP expression levels [62]. Finally, RKIP overexpression induced by Snail knockdown has been shown to increase apoptosis rates, reverse EMT, and reduce the expression of cancer stem cell (CSC) markers in colorectal cancer, contributing to the reversal of chemoresistance [64].

In summary, the RKIP inhibitory role on the NF-kB/YY1/Snail axis leads to the suppression of cell migration, ETM and metastasis, with the concomitant restoration of the apoptotic sensitivity counteracting tumor chemoresistance mediated by the dysregulation of NF-kB signaling [65–68].

2.1.3. RKIP regulation of the G protein-coupled receptor (GPCR) pathway

RKIP plays a role as a homeostatic sensor in response to extracellular stimuli, not only by directly modulating MAPK cascade signaling but also by activating GPCR pathway [34]. Indeed, the GPCR-induced activation of the Protein Kinase C (PKC) determines the phosphorylation of RKIP at Serine 153 (p-Ser153 RKIP), resulting in the release of Raf-1 and the subsequent activation of the MAPK pathway [5,25]. Furthermore, RKIP phosphorylation facilitates its interaction with G protein-coupled receptor kinase 2 (GRK2), a negative regulator of GPCRs by promoting their internalization. RKIP-GRK2 interaction leads to the dissociation of GRK2 from GPCRs, thereby activating GPCR signaling [25]. The activation of this pathway results in the phosphorylation and activation of downstream targets, including Raf-1. Therefore, depending on the phosphorylation status, RKIP can indirectly modulate Raf-1, thus the entire MAPK cascade, by interfering with the regulation of its upstream activators, GPCRs.

In contrast to the unphosphorylated RKIP, p-Ser153 RKIP inhibits cancer cell growth and enhances apoptosis. Numerous studies have demonstrated that elevated levels of p-Ser153 RKIP correlate positively with patients' survival and less aggressive tumors. Thus, the overexpression of p-Ser153 RKIP represents a positive prognostic factor of good prognosis in various cancer types [69–71].

2.1.4. RKIP regulation of GSK3β

The RKIP/GSK3β interaction has been reported to promote GSK3β stabilization and activation, preventing its inhibitory phosphorylation at the T390 residue [9].

In cancer cells, RKIP depletion favors cell migration and EMT by disrupting its interaction with GSK3β, a tumor suppressor involved in the regulation of Wnt and Cyclin D pathways.

RKIP downregulation leads to high levels of oxidative stress, which activates p38 MAPK. Activated p38 MAPK mediates the inactivation of GSK3β through phosphorylation at the C-terminus region, specifically at T390 [72]. Once GSK3β-mediated suppression is removed, its downstream targets are activated. Thus, the stabilization of cyclin D1 leads to cell cycle progression, while the expression of β-catenin, Snail and SLUG contributes to enhanced cell invasion and EMT [9].

2.1.5. RKIP regulation of STAT3 signaling

The signal transducer and activator of transcription 3 (STAT3) protein is a pro-oncogenic transcription factor often found to be constitutively activated in various human tumors [73]. Its phosphorylated activation has been associated with tumor progression, promoting cell growth, EMT, migration, and metastasis [74].

Studies performed on different cancer types have demonstrated that RKIP expression was inversely correlated with phosphorylated STAT3 (pSTAT3) levels, highlighting their putative prognostic value when dysregulated [7,8,26,75,76]. RKIP exerts direct and indirect mechanisms to prevent STAT3 activation. In particular, it was demonstrated that RKIP overexpression determines the suppression of IL-6 and, consequently, blocks the IL-6-mediated STAT3 phosphorylation [26,75]. Moreover, the inhibition of the STAT3 pathway mediated by RKIP may also occur through a direct RKIP-STAT3 interaction. Indeed, it was demonstrated that exogenous overexpression of RKIP is associated with increased RKIP-STAT3 interaction and a concomitant reduction of nuclear pSTAT3 level, indicating the inhibitory role of RKIP on STAT3. Thus, through direct and indirect regulation, RKIP inhibits the transcriptional activity of STAT3, resulting in the suppression of cell migration in cancer cells [8,75].

2.2. Regulation of RKIP expression

Despite its central role in the regulation of several cellular and molecular pathways, RKIP is finely regulated at multiple levels, including transcriptional, epigenetic, post-transcriptional, and post-translational, through complex mechanisms that contribute to tumor progression [77]. These diverse mechanisms of control enable cancer cells to fine-tune RKIP activity, promoting oncogenic progression, metastasis, and therapy resistance [66] (Fig. 2).

Therefore, understanding the mechanisms regulating RKIP is essential not only for elucidating its role in tumor biology but also for identifying novel diagnostic and prognostic biomarkers, as well as potential

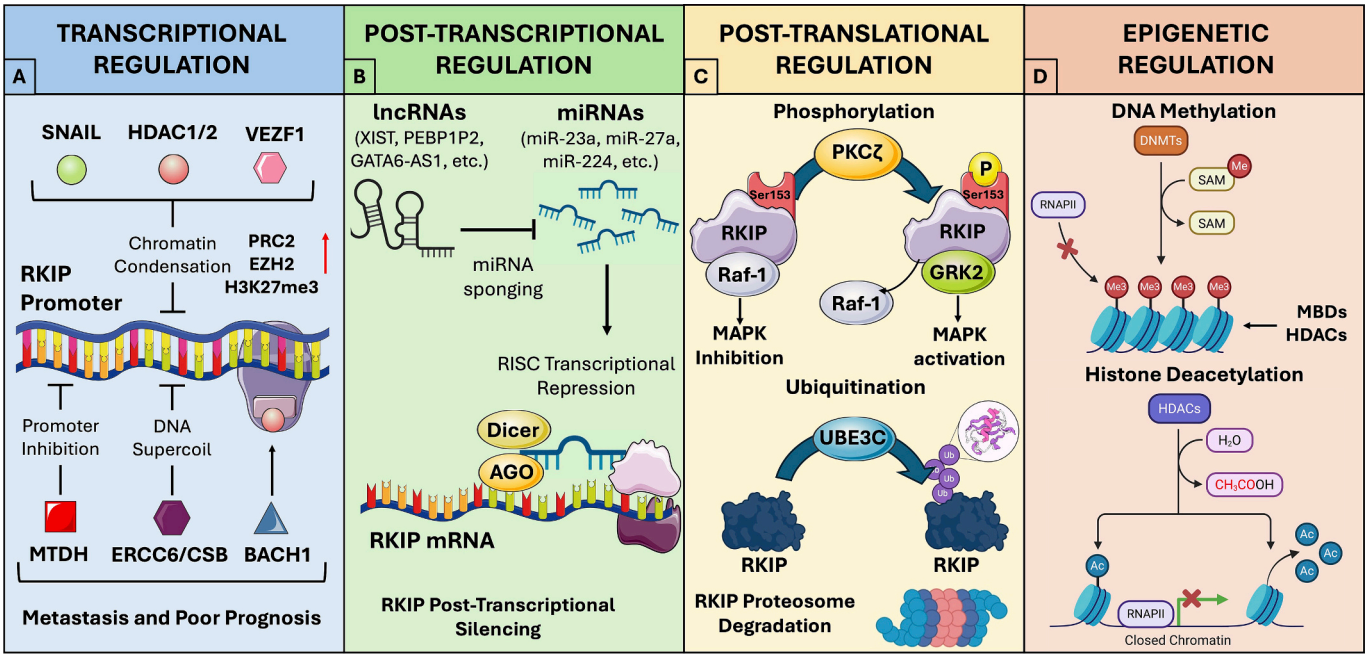

Fig. 2. Molecular mechanisms responsible for RKIP expression in cancer. A) Transcriptional regulation is mainly mediated by several transcription factors able to bind the RKIP promoter and induce chromatin condensation, promoter inhibition and DNA supercoil, finally leading to metastasis and poor prognosis; B) RKIP post-transcriptional regulation is mainly mediated by lncRNAs able to sponge miRNAs associated with RKIP regulation and miRNAs themselves able to bind RKIP through the RISC complex inducing its post-transcriptional silencing; C) At the post-translational level, PKCζ phosphorylates RKIP, inducing the activation of MAPK signaling, while RKIP degradation is mediated by UBE3C ubiquitination and proteosome degradation; D) The epigenetic regulation of RKIP is both mediated by histone deacetylation and DNA methylation that influence chromatin structure and transcriptional accessibility, respectively.

therapeutic targets aimed at restoring its tumor-suppressive functions.

In particular, the discovery of epigenetic modulators such as transcriptional repressors, chromatin remodeling factors, and miRNAs opens new avenues for cancer treatment aimed at reactivating silenced tumor suppressors [19].

2.2.1. *Transcriptional regulation of RKIP*

The transcriptional repression is one of the primary mechanisms responsible for the loss of RKIP expression in cancer. Several transcription factors have been identified as negative regulators of the RKIP promoter, either directly binding to its regulatory regions or acting via recruitment of repressive chromatin-modifying complexes [78].

One of the most studied repressors is Snail (SNAI1), a master regulator of EMT. Snail directly binds to *E*-box elements within the RKIP promoter and recruits chromatin modifiers such as the Polycomb Repressive Complex 2 (PRC2), of which EZH2 is the catalytic subunit responsible for trimethylation of histone H3 at lysine 27 (H3K27me3). This process facilitates chromatin compaction and transcriptional silencing of RKIP [79]. Snail has also been shown to cooperate with histone deacetylases (HDAC1/2), further reinforcing repression via deacetylation of histone tails, thereby contributing to a closed chromatin configuration [21,80].

Another transcriptional repressor implicated in RKIP down-regulation is BTB and CNC homology 1 (BACH1), which similarly targets the RKIP promoter and interacts with PRC2 and HDAC complexes. BACH1-mediated repression of RKIP has been reported particularly in prostate cancer and is associated with enhanced metastatic potential and poor prognosis [81].

Metadherin (MTDH) is another oncogenic transcriptional regulator that represses RKIP expression. Chromatin immunoprecipitation (ChIP) assays have demonstrated direct binding of MTDH to the RKIP promoter in breast cancer cells [82]. Silencing of MTDH results in the upregulation of RKIP and suppression of metastasis-related genes, suggesting that MTDH contributes to metastatic progression, at least in part, by silencing RKIP [82].

Two additional transcription factors, vascular endothelial zinc finger 1 (VEZF1) and excision repair cross-complementation group 6 (ERCC6/CSB), have been implicated in the transcriptional control of RKIP in prostate cancer. VEZF1 is thought to regulate RKIP expression during chromatin remodeling events, although the precise binding dynamics remain to be fully elucidated. ERCC6, a DNA-dependent ATPase involved in transcription-coupled repair, may influence RKIP expression indirectly through alterations in chromatin structure and transcription elongation [82,83].

Collectively, these transcriptional repressors downregulate RKIP through both epigenetic and chromatin-based mechanisms. Their activity not only facilitates tumor progression and metastasis but also contributes to therapy resistance, making them potential targets for epigenetic therapy aimed at restoring RKIP expression (Fig. 2A).

2.2.2. *Epigenetic regulation of RKIP*

Epigenetic silencing is a key mechanism underlying the loss of RKIP/PEBP1 expression in cancer. Unlike genetic mutations, epigenetic alterations are reversible and involve heritable changes in gene expression without altering the DNA sequence [84]. These modifications primarily include DNA methylation of promoter CpG islands and post-translational histone modifications, both of which influence chromatin structure and transcriptional accessibility [19]. Understanding the epigenetic regulation of RKIP is not only crucial for elucidating cancer progression mechanisms but also opens avenues for targeted epigenetic therapies, especially considering the reversibility of these modifications (Fig. 2D).

As regards promoter methylation, the hypermethylation of cytosine residues within CpG islands located in the promoter region of the RKIP has been reported as a frequent mechanism of its transcriptional repression in multiple cancer types [85]. This modification is mediated by DNA methyltransferases (DNMTs), which catalyze the addition of a methyl group to the 5′ position of cytosine residues in CpG dinucleotides [86].

In normal cells, the RKIP promoter is typically unmethylated,

allowing active transcription and its tumor suppressor activities [87]. However, in tumor cells, de novo methylation leads to transcriptional silencing by recruiting methyl-CpG-binding domain proteins (MBDs) and associated repressor complexes, including histone deacetylases (HDACs) and co-repressors promoting a closed chromatin conformation, impairing RNA polymerase II recruitment and effectively shutting down gene expression [86,88].

Several studies using methylation-specific PCR (MSP), bisulfite sequencing, and pyrosequencing have confirmed the presence of hypermethylation phenomena affecting the RKIP promoter in patient-derived tumors and cancer cell lines [88]. Specifically, in gastric cancer, a strong inverse correlation has been found between RKIP promoter methylation and mRNA/protein levels, proposing methylation as a driver phenomenon responsible for functional silencing [87,89]. Similarly, in breast cancer, RKIP promoter methylation has been associated with triple-negative subtypes and poor prognosis, suggesting its potential use as a potential epigenetic biomarker for this pathology [19,90]. In this regard, promoter methylation may serve as a diagnostic or prognostic biomarker, suggesting how the evaluation of RKIP methylation levels in tumor biopsies or even in circulating DNA from liquid biopsies may be proposed as a non-invasive strategy for cancer detection or monitoring [77,91].

Other findings demonstrated that the methylation-mediated silencing of RKIP in CRC and esophageal cancer has been linked to increased invasiveness and Wnt pathway activation, further corroborating its role in CRC progression [85,87,92,93].

Since promoter methylation significantly impacts RKIP expression, some researchers started to investigate the potential therapeutic effects of DNA demethylating agents on cancer cells. The use of 5-aza-2′-deoxycytidine (5-aza-dC) or decitabine has been shown to reactivate RKIP expression, reduce proliferation, and restore sensitivity to chemotherapy and apoptosis in preclinical models [94–96].

As regards histone modifications, chromatin structure is also dynamically regulated by covalent modifications of histone tails, including acetylation, methylation, and phosphorylation, which impact gene accessibility [97]. In the case of RKIP, trimethylation of histone H3 at lysine 27 (H3K27me3), a well-established transcriptional repressive mark, has been identified as a major epigenetic modification associated with gene silencing [19].

As already mentioned, EZH2 is frequently overexpressed in aggressive tumors and is also responsible for catalyzing H3K27me3 deposition at the RKIP promoter, resulting in a condensed chromatin state that prevents RKIP transcriptional initiation [19]. By cooperating with HDAC1/2, EZH2 further enhances chromatin compaction and transcriptional repression through the removal of acetyl groups from histone tails [80,98].

Pharmacological inhibition of EZH2 (e.g., tazemetostat) or HDACs (e.g., vorinostat, trichostatin A) has been shown to partially reactivate RKIP expression, thereby sensitizing tumor cells to chemotherapy and reducing their invasive potential [99,100]. These findings suggest that targeting chromatin modifiers may represent a promising therapeutic strategy to restore RKIP function in cancer.

2.2.3. *Post-translational modification of RKIP*

While transcriptional and epigenetic mechanisms significantly influence RKIP expression, post-translational modifications (PTMs) play a crucial role in modulating its activity, stability, and protein–protein interactions. These modifications fine-tune RKIP function in response to intracellular signals, often shifting its role from a tumor suppressor to an inert or mislocalized protein in cancer cells [70,77].

One of the most studied PTMs affecting RKIP is phosphorylation, particularly at the conserved serine residue Ser153. This modification, mainly mediated by protein kinase C zeta (PKCζ), induces a functional shift in RKIP by causing its dissociation from Raf-1 kinase [25,101]. As mentioned before, in its unphosphorylated state, RKIP binds Raf-1 and inhibits the Raf/MEK/ERK signaling cascade, thereby exerting antiproliferative effects. Upon phosphorylation, RKIP loses its ability to inhibit this pathway and instead binds to G protein-coupled receptor kinase 2 (GRK2), modulating GPCR signaling through the regulation of receptor desensitization and internalization. This switch reprograms cellular signaling, promoting mitogenic and survival pathways, and has been associated with increased tumor aggressiveness in several cancer types, including prostate and breast cancer [100].

Notably, elevated levels of phosphorylated RKIP, even in the presence of total protein, correlate with poor clinical prognosis in multiple cancers, underscoring the importance of functional inactivation through PTMs rather than simple downregulation of gene expression [75,81,102].

Ubiquitination represents another mechanism of PTM that contributes to RKIP silencing in a subset of tumors. Recent findings in clear cell renal cell carcinoma have identified the E3 ubiquitin ligase UBE3C as a key mediator of RKIP degradation. This process is orchestrated through a novel axis involving circPOLR2A, a circular RNA that enhances UBE3C expression and facilitates its nuclear translocation. Once active, UBE3C targets RKIP for polyubiquitination and subsequent degradation via the proteasome pathway. The loss of RKIP protein leads to sustained activation of the ERK pathway, driving cell proliferation, invasion, and metastasis [3,103].

The axis mediated by RKIP, ubiquitin and NF-κB pathway was also described in prostate cancer as a contributor to cancer progression and inhibition [60]. These findings highlight a cancer-specific, non-genomic strategy of RKIP silencing that operates independently of transcriptional repression or promoter methylation.

Altogether, PTMs add a critical layer of complexity to the control of RKIP function in cancer. By altering RKIP localization, interaction partners, or protein stability, cancer cells can dynamically adjust intracellular signaling to favor growth, survival, and dissemination. From a therapeutic standpoint, the reversibility and druggability of these modifications present unique opportunities for restoring RKIP function in malignancies characterized by its functional inactivation (Fig. 2C).

2.2.4. *microRNA-mediated regulation of RKIP*

Among the various mechanisms controlling RKIP expression and function, miRNAs have emerged as pivotal post-transcriptional regulators. Their ability to finely regulate RKIP levels with high specificity and responsiveness to cellular context makes them central players in cancer progression. Given their emerging significance, this review will primarily focus on miRNA-mediated regulation of RKIP and the next sections will explore in detail the diverse miRNAs implicated in RKIP regulation and their impact on tumor biology and therapeutic resistance in multiple cancers (Fig. 2B).

3. miRNA-RKIP interaction in cancer

To elucidate the miRNA-RKIP interaction in cancer, a comprehensive literature search was conducted by screening PubMed, Web of Science, and Scopus databases to investigate the role of microRNAs involved in the RKIP regulation in multiple cancers. The literature search included papers published until May 2025.

Specific keywords in combination with the Boolean operator were used to maximize the sensitivity of the search query, as follows: "(((RKIP) OR (PEBP1)) AND (microRNA) AND (cancer))". The lists of articles obtained from the different databases were combined, and duplicates were manually eliminated. The merged list of articles was screened by two independent authors, at first examining only titles and abstracts; afterwards, the screened articles were extensively reviewed, by reading the full text, to assess the suitability of the selected articles, according to the eligibility criteria established a priori (Fig. 3).

Inclusion criteria consisted of selecting articles aimed at investigating the role of microRNAs in mediating the RKIP regulation, with no restriction applied about cancer types; however, only articles showing

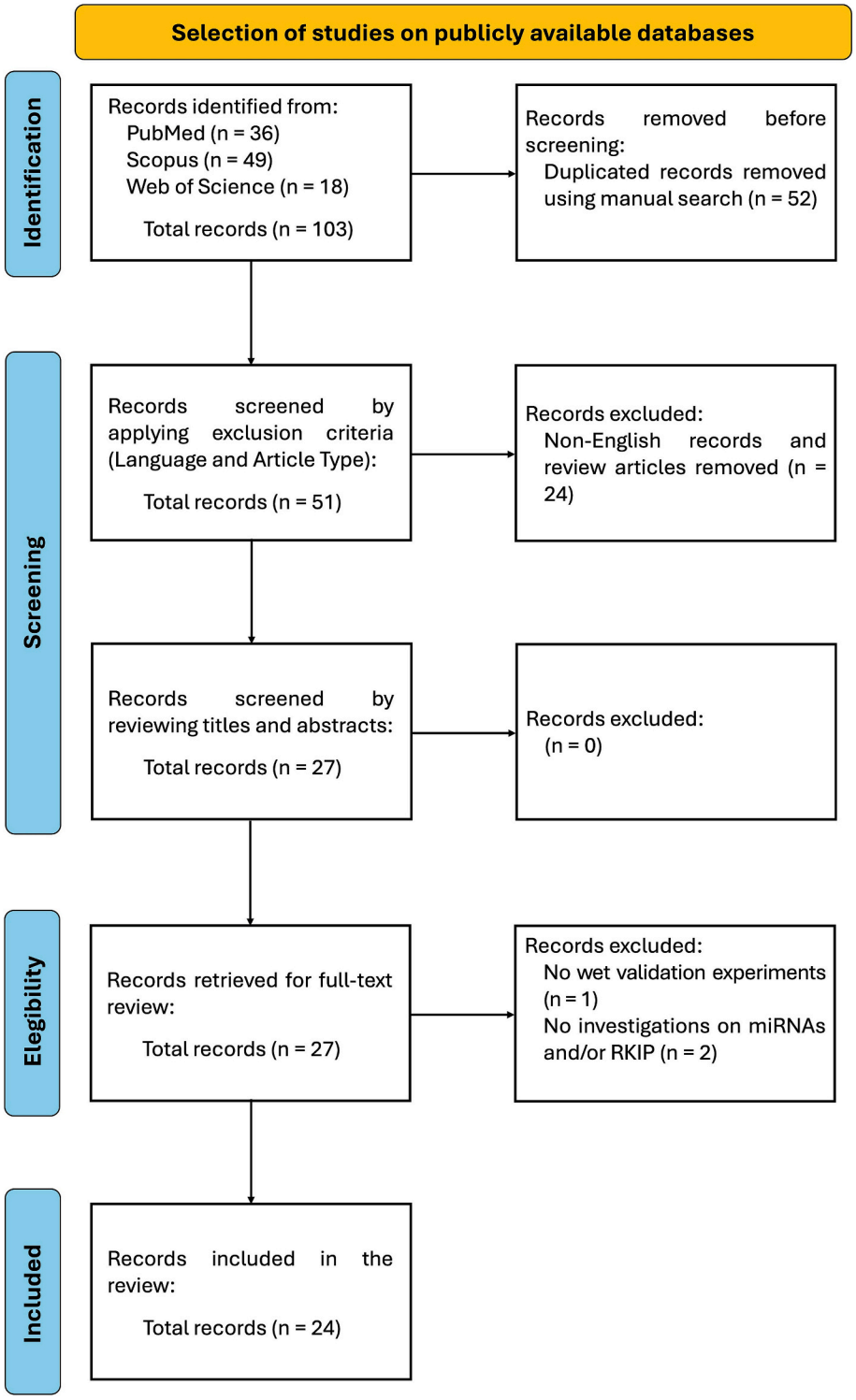

Fig. 3. Algorithm for the selection of studies to be included in the review.

consistency of results, obtained through functional wet validation studies, were included.

As regards the exclusion criteria, articles reporting the association between microRNAs and RKIP based only on bioinformatics analyses or prediction investigations, as well as literature reviews, were excluded. Additionally, articles not published in English, those with no available full text, and retracted articles were not considered.

Finally, the resulting selected articles were summarized, and the relevant findings were extracted and analyzed by grouping them according to the type of tumor.

The literature search resulted in the selection of several studies demonstrating that the expression of RKIP is frequently regulated at the post-transcriptional level by miRNAs across a wide range of malignancies (Table 1) [14,19,23,24,94,95,102,104–120].

The next sections will explore, in a tumor-specific manner, the molecular mechanisms through which distinct miRNAs modulate RKIP expression, thereby contributing to oncogenic processes such as uncontrolled proliferation, invasion, metastasis, and therapy resistance.

Table 1
Studies investigating the interaction between RKIP and miRNAs in multiple tumors.

Tumor type	Tumor Model(s)	microRNA	microRNA Target	RKIP regulatory mechanism	Functional effects	Validation assays	Ref
Breast cancer	MDA-MB-231, 1833 and 4175 metastatic breast cancer cell lines. BALB/c female nude mice	let-7	HMGA2	RKIP downstream effector	Suppression of cell invasion via the MAPK/Myc/LIN28/let-7/HMGA2 axis: RKIP downregulates Myc and LIN28, leading to upregulation of let-7, which reduces HMGA2 expression.	In vitro cell invasion and cell proliferation assays, qRT-PCR, Immunoblotting, ChIP analysis, and luciferase reporter assay	[14]
	MDA-MB-2311833, MDA-MB-436, MDA-MB-435 breast cancer cell lines. BALB/c female nude mice	let-7	BACH1	RKIP downstream effector	Suppression of metastasis formation mediated by the RKIP/let-7 axis. RKIP upregulates let-7, which targets and downregulates BACH1. BACH1 downregulation suppresses the expression of metastasis-related genes MMP1, CXCR4, and OPN.	In vitro cell invasion and cell proliferation assays, Luciferase reporter assay, Immunoblotting, qRT-PCR, ChIP analysis	[104]
	T47D, MCF7, and MDA-MB-231 breast cancer cell lines	miR-101	EZH2	RKIP upstream regulator (transcriptional regulation)	Suppression of cell invasion through transcriptional regulation of RKIP indirectly mediated by miR-101, which targets and represses EZH2, a histone methyltransferase that suppresses RKIP transcription. Loss of miR-101 leads to EZH2 overexpression, resulting in RKIP downregulation and increased metastatic potential.	qRT-PCR, Western blot analysis, ChIP analysis, In vitro invasion assay and Luciferase reporter assay	[19]
	MCF-10A, MDA-MB-468, T47D, MCF-7, MDA-MB-453, MDA-MB-231 breast cancer cell lines	miR-224	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Modulation of cancer cell invasion and metastasis promotion through miR-224-mediated suppression of RKIP. RKIP downregulation leads to increased expression of prometastatic genes, MMP1, OPN, and CXCR4.	qRT-PCR, Western blot analysis, Three-dimensional spheroid invasion assay, Transwell matrix penetration assay, Wound healing assay and Luciferase reporter assay	[105]
	MDA-MB-2311833, MDA-MB-436, MDA-MB-435 breast cancer cell lines. BALB/c female nude mice.	miR-200b	LOX	RKIP downstream effector	Regulation of extracellular matrix remodeling and metastasis suppression via RKIP/HMGA2/miR-200b/LOX axis: RKIP downregulates HMGA2, which in turn increases miR-200b levels. miR-200b directly targets and inhibits LOX, thus reducing tumor invasiveness.	Gene and microRNA expression array analyses, qRT-PCR, Immunoblotting, In vitro cell proliferation, apoptosis and invasion assays, In vivo tumor growth and bone metastasis assays, Luciferase reporter assay, Immunostaining	[106]
	BT549, MCF-7, MCF-7R, MDAMB-231, MDA-MB-468, SUM159 and SUM149 breast cancer cell lines	miR-224	–	–	No correlation between miR-224 and RKIP expression in triple-negative breast cancer: miR-224 does not influence RKIP expression. RKIP suppression is due to promoter hypermethylation, histone deacetylation, and NF-κB activation.	In vitro cell growth and cell invasion assays, Flow cytometry-based cell death assay, Immunohistochemical analysis, Methylation-specific PCR, qRT-PCR, Western blotting analysis.	[94]
	Human breast cancer and normal breast tissue samples, Hs578T, MCF-7, MDA-MB-231 and MDA-MB-453 breast cancer cell lines	miR-185	HMGA2	RKIP downstream effector	Modulation of cancer cell growth and invasion via RKIP/miR-185/HMGA2 axis. RKIP positively regulates miR-185, which in turn directly targets HMGA2, inhibiting cell proliferation and invasion.	qRT-PCR, Western blot analysis, BrdU incorporation assay, Cell invasion assay.	[107]
Prostate cancer	Prostate cancer tissue samples, DU-145, PC-3 and LNCaP prostate cancer cell lines.	miR-101	EZH2	RKIP upstream regulator (transcriptional regulation)	Promotion of cancer cell invasion through transcriptional regulation of RKIP indirectly mediated by miR-101, whose target is EZH2, a histone methyltransferase that suppresses RKIP transcription. The downregulation of miR-101 promotes EZH2 expression, which leads to	qRT-PCR, Western blot analysis, ChIP analysis, In vitro invasion assay, Luciferase activity assay, Immunohistochemistry analysis.	[19]

(*continued on next page*)

Table 1 (*continued*)

Tumor type	Tumor Model(s)	microRNA	microRNA Target	RKIP regulatory mechanism	Functional effects	Validation assays	Ref
	Prostate cancer and adjacent normal tissue samples PC3, DU145 and LNCAP prostate cancer cell lines RWPE1 human prostate cells. BALB/C nude mice.	miR-23a	RKIP	RKIP direct regulation, (post-transcriptional regulation)	RKIP suppression and cancer progression and metastasis. Cancer cell invasion and RKIP post-transcriptional regulation mediated by the XIST/miR-23a/RKIP axis: miR-23a directly targets and downregulates RKIP, while the lncRNA XIST acts as a molecular sponge to sequester miR-23a, indirectly increasing RKIP expression levels.	qRT-PCR, In vitro cell proliferation assay, cell invasion assay, cell cycle assay, wound healing assay, Luciferase reporter assay, RNA immunoprecipitation assay, Western blot assay, Tumor xenograft formation assay, Immunohistochemistry analysis.	[108]
	Prostate cancer and adjacent normal tissue samples LNCaP and C4—2B prostate cancer cell lines. BALB/c nude mice.	miR-543	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Promotion of cell proliferation, invasion, and EMT by miR-543-mediated suppression of RKIP.	Luciferase reporter assays, qRT-PCR, Western blot analysis, Cell proliferation assay, Cell invasion and migration Assays, Cell cycle assay, Immunohistochemistry analysis.	[109]
	NSCLC tissue samples, 549 NSCLC cells, female BALB/c nude mice.	miR-27a	RKIP	RKIP direct regulation (post-transcriptional regulation)	EMT and chemoresistance induction due to upregulation miR-27a, which directly targets and suppresses RKIP expression levels.	qRT-PCR, Western blot analysis, Cell viability assay, Transwell invasion assay, Luciferase reporter assay.	[110]
	NSCLC and normal tissue samples, H460, A549, H1299, 95C, 95D NSCLC cell lines, MRC-5 human normal lung cells, Nude mice.	miR-150	FOXO4	RKIP upstream regulator (transcriptional regulation)	Promotion of metastasis and EMT via FOXO4/NF-κB/Snail axis, inducing the indirect RKIP suppression. miR-150 targets and suppresses FOXO4, resulting in the activation of the NF-κB/Snail pathway and, consequently, in the Snail-mediated transcriptional repression of RKIP.	qRT-PCR, Wound-healing assay, Transwell migration assays, Western blot analysis, F-actin staining, Dual luciferase reporter assay, Soft agar colony formation assay, in vivo tumorigenicity and tumor metastasis assays, Immunohistochemistry.	[111]
Lung cancer	NSCLC and normal tissue samples, A549, 95-D, H1299, H292, and H460 NSCLC cell lines, BEAS-2B normal lung epithelial cells. Female BALB/c nude mice.	miR-543	RKIP	RKIP direct regulation (post-transcriptional regulation)	Modulation of tumor proliferation, invasion, and EMT mediated by the GATA6-AS1/miR-543/RKIP axis. miR-543 directly suppresses RKIP expression in NSCLC, while the lncRNA GATA6-AS1 sponges miR-543, thereby restoring RKIP levels.	qRT-PCR, Viability assay, Transwell Migration and Invasion assay, Western blot analysis, Luciferase reporter Assay.	[112]
	Clinical specimens, A549, and PC9 LUAD cell lines, BEAS-2B normal lung epithelial cells.	miR-3940-5p	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Promotion of tumor progression mediated by miR-3940-5p. The upregulation of miR-3940-5p in lung adenocarcinoma inversely correlates with RKIP expression and it is associated with poor prognosis and advanced stages.	qRT-PCR	[113]
	MGC80–3, SGC-7901, and MKN45 gastric cancer cell lines, GES-1 normal gastric cells	miR-224	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Increased cell proliferation and invasion, and anti-apoptotic effect promoted by miR-224-mediated inhibition of RKIP	qRT-PCR, flow cytometric-based apoptosis analysis, viability assay, Transwell migration assay, Luciferase reporter Assay, Western blot assay.	[114]
Gastric cancer	Gastric cancer and normal tissue samples, BGC823 and SGC7901 gastric cancer cell lines, GES-1 normal gastric cells, Human leukemia monocytic U937 cells, female BALB/c nude mice.	miR-27a, miR-155	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Promotion of cell proliferation, cell invasion, metastasis and chemoresistance via Bmi-1/miR-27a/miR-155/RKIP axis: Bmi-1 induce miR-27a and miR-155 to directly target and suppress RKIP.	miRNA microarray analysis, Luciferase reporter assay, RNA electrophoretic mobility shift assays, Tumor xenograft formation assay, Immunohistochemistry analysis.	[115]
	Gastric cancer and normal tissue samples, MKN-45, MGC-803, and AGS gastric cancer cell lines, GES-1 normal gastric cells.	miR-3200-5p	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Regulation of cell proliferation, invasion, and EMT mediated by the circMTO1/miR-3200-5p/RKIP axis, where circMTO1, sponging miR-3200-5p, mitigates its inhibitory effect on RKIP.	qRT-PCR, Cell proliferation assay, Transwell assay, Colony formation assay, Western blot analysis, Fluorescence in situ hybridization, Luciferase reporter assay.	[116]
Liver cancer	Liver cancer and normal tissue samples, HA22T/VGH, HepG2, and Hep3B	miR-224	–	–	miR-224 does not regulate RKIP expression in the examined HCC cell lines, as	qRT-PCR, Western blotting, Methylation-specific polymerase	[95]

(*continued on next page*)

Table 1 (*continued*)

Tumor type	Tumor Model(s)	microRNA	microRNA Target	RKIP regulatory mechanism	Functional effects	Validation assays	Ref
	hepatocellular carcinoma cell lines.				expected from in silico analyses and previous studies. The presence of the pseudogene PEBP1P1 likely acts as a miRNA decoy sequestering.	chain reaction, Immunohistochemical analysis,	
	Liver cancer and normal serum samples, A549 cell line and MCC-7721 hepatocellular carcinoma cells	miR-27a	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Promotion of cell proliferation, EMT, cell migration, and cisplatin resistance by miR-27a-mediated suppression of RKIP.	qRT-PCR, cell proliferation assay, Luciferase reporter assay, Western blot analysis.	[117]
	HepG2 and HCC340 hepatocellular carcinoma cell lines	miR-6134	CCNE1	–	Regulation of cell proliferation and migration through the dysregulation of MAPK pathway mediated by RKIP and miR-6134, which act in concert to regulate TPA-induced responses.	Transwell migration assay, LC-MS/MS analysis, qRT-PCR, Immunoprecipitation and Western blot analysis, PKC kinase activity determination, Confocal immunofluorescence analysis, Analysis of cell cycle, Mitochondrial ROS generation assay, RNAseq and microRNA-microarray.	[102]
	Glioma and normal brain tissue samples, U251, U87, and SHG44 glioma cell lines, HEB human normal glial cells	mir-98	HMGA2	RKIP downstream effector	Regulation of cell invasion mediated by RKIP/miR-98/HMGA2 axis: RKIP positively regulates miR-98 expression, which in turn suppresses the pro-metastatic gene HMGA2.	qRT-PCR, Western Blot Analysis, BrdU Incorporation Assay, Cell Invasion Assay.	[118]
	Acute Myeloid Leukemia clinical samples, THP-1, U937, and NB4 AML cell lines, HEK-293 normal cells	miR-23a	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Increased cell proliferation through the miR-23a-mediated suppression of RKIP expression	qRT-PCR, Immunoblot analysis, Luciferase reporter assays, cell growth and proliferation analyses.	[23]
Other tumors	Nasopharingeal carcinoma and normal tissue samples, CNE2 and CNE2-IR nasopharingeal carcinoma cell lines, nude male mice.	miR-181a	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Enhancement of radioresistance through the miR-181a-mediated suppression of RKIP	qRT-PCR, cell viability and survival assays, Flow Cytometric apoptosis assay, Western Blot analysis, Dual-Luciferase reporter assay, In vivo tumor radioresponse assay, Immunohistochemistry.	[24]
	Pancreatic cancer and normal tissue samples, PANC-1 pancreatic cancer cell line	miR-181a	RKIP	RKIP direct regulation, (post-transcriptional regulation)	Promotion of cell migration, cell invasion, and EMT through the miR-181a-mediated downregulation of RKIP.	Western blot analysis, Sphere growth and invasion assay, qRT-PCR, Immunofluorescence analyses.	[119]
	HEK293T, HK-2, ACHN, A-498, and 786-O renal carcinoma cell lines, 6-week-old NCG mice	miR-296, miR-616, and miR-3194	RKIP, KLF13	Indirect regulation of cell cycle through independent mechanism	Regulation of cell migration and invasion mediated by PEBP1P2, which sponges oncogenic miR-296, miR-616, and miR-3194. Silencing PEBP1P2 decreases RKIP and KLF13 levels.	qRT-PCR, ChIP assay and dCas9-ChIP assay, Dual-Luciferase reporter assay, RNA immunoprecipitation (RIP) and MS2-RIP assay, RNA pulldown assays, Western blot analysis, Fluorescence in situ hybridization, Immunohistochemistry, Transwell assay.	[120]

Each subsection focuses on a specific cancer type, including breast, prostate, lung, gastric, liver, and other solid and hematological malignancies, highlighting key experimental findings and elucidating the complex regulatory networks involved. This comprehensive analysis not only underscores the central role of miRNA-mediated RKIP suppression in tumor progression but also sheds light on potential therapeutic strategies aimed at restoring RKIP function by targeting its upstream non-coding RNA regulators.

3.1. Breast cancer

Variation in RKIP expression levels has been widely associated with breast cancer aggressiveness by multiple studies. The downregulation of RKIP or its loss is frequently associated with metastatic and more invasive tumor phenotypes, confirming the metastasis suppressor role of RKIP in breast cancer [121]. In addition, RKIP plays a pro-apoptotic function in breast cancer cells by sensitizing them to drug-induced apoptosis [63]. Among the numerous pathways regulated by RKIP, it seems to mediate metastasis suppression by modulating pathways involved in the immune response in breast cancer [122]. Since RKIP alterations have been observed at low frequency in breast cancer, the loss of RKIP may be due to post-transcriptional mechanisms [121]. In this context, a consistent literature demonstrates the involvement of microRNA in the regulation of RKIP expression in breast cancer.

Dangi-Garimella and colleagues (2009) investigated the role of RKIP in inhibiting breast cancer cell invasion by performing both in vitro and in vivo experiments, particularly focusing on its role in the modulation of MAPK signaling. For this purpose, they used highly invasive triple-negative breast cancer cells (MD-MBA-231), expressing low levels of RKIP. By restoring the RKIP levels through the transfection of lentiviral

vectors expressing wild-type and S153E mutant RKIP, they demonstrate the inhibition of invasion in both transfected clones, without any perturbation on E-cadherin and vimentin expression levels. Since the phosphorylation of RKIP at the S153 site leads to its dissociation from Raf-1 and, consequently, to the activation of the MAPK pathway, in the S153 mutant cells, RKIP cannot be phosphorylated and maintains its binding with Raf-1, thus inhibiting MAPK signaling.

By injecting the MDA-MB-231 bone tropic subclone (1833), transfected with wt RKIP or S153E RKIP into the mammary fat pad of a murine orthotopic model, the authors also showed a significant decrease in bone metastasis formation compared to controls. In order to understand the mechanism underlying the metastasis suppression role of RKIP, they studied the signaling cascade involving MAPK, LIN28, *let-7,* and its downstream targets. In particular, the inhibition of RKIP led to the downregulation of LIN28 mediated by Myc, which in turn promotes the upregulation of *let-7,* responsible for the downregulation of HMGA2 and Snail, and the inhibition of cell invasion and metastasis suppression. Conversely, the use of a *let-7* anti-miR restored HMGA2 and Snail expressions and the promotion of cell invasion. Finally, the authors further investigated this post-transcriptional regulatory axis, demonstrating that RKIP acts as a metastasis suppressor by increasing let-7 expression through LIN28 inhibition. In this axis, RKIP is also responsible for the suppression of the ability of Myc to promote LIN28 transcription. Therefore, the entire Myc/LIN28/let-7 axis may be regulated upstream by the activation of MEK, which upregulates Myc and LIN28, promoting cell invasion, while MEK inhibition reduces Myc and LIN28, suppressing invasion [14].

The role of RKIP regulation in breast cancer metastasis mediated by *let-7* continued with the investigations of Yun J and colleagues (2011), which identified BACH1 as a novel *let-7* target involved in the RKIP/let-7 metastasis pathway. Starting from a computational analysis performed on breast cancer gene expression datasets obtained from the GEO repository (BrCa443, BrCa871), they identified a candidate list of high-confidence *let-7* targeted genes, found downregulated in tumors with higher RKIP levels. Among these genes, the authors focused on leucine zipper transcription factor BACH1. Moreover, the RKIP/*let-7* axis was predicted to regulate bone metastasis genes, MMP1, CTGF, OPN and CXCR4. In this molecular landscape, BACH1 was identified as a direct transcriptional regulator of the bone metastasis gene MMP1. The in vitro studies performed on RKIP wild-type and RKIP S153E mutated 1833 cells revealed a downregulation of both BACH1 and HMGA2 mRNA. By using a mutant BACH1 3' UTR luciferase reporter construct, they observed that *let-7*-expressing cells do not inhibit the mutated BACH1 3' UTR, demonstrating that BACH1 is a direct target of *let-7*. Furthermore, the RKIP-expressing cells and the transfected *let-7* cells showed a downregulation of bone metastasis genes MMP1, CXCR4 and OPN. Specifically, the depletion of BACH1 decreased MMP1 and CXCR4 levels, inhibiting invasion but not cell proliferation. Since the promoter region of MMP1 showed two potential BACH1 sites, by mutating these MMP1 promoter regions, the authors confirmed that MMP1 is a transcriptional target of BACH1. In vivo investigations were performed on immunocompromised mice by using 1833 cells silenced or overexpressing the investigated genes. At first, they showed that the depletion of BACH1 and HMGA2 decreased the bone metastasis, while the overexpression of HMGA2 restored metastasis formation. Furthermore, BACH1 depletion also inhibited intravasation, but not primary tumor growth, confirming the role of BACH1 in regulating invasion, intravasation, and metastasis formation.

Finally, the analysis of genes involved in bone metastasis showed that combined overexpression of MMP1, OPN, and/or CXCR4 reduced the anti-metastatic effects of RKIP and partially reversed the invasion-inhibiting role played by *let-7*. Integrating these findings, the authors identified a metastasis regulation pathway where RKIP inhibits BACH1, which in turn suppresses the breast cancer metastasis genes MMP1, OPN, and CXCR4 [104].

Another study by Ren G and colleagues (2012) highlighted the regulatory role of microRNAs, particularly miR-101, in modulating RKIP expression through their effects on EZH2, a histone methyltransferase that promotes cancer aggressiveness. miR-101 expression was found to be downregulated in breast cancer, and this loss contributes to the overexpression of EZH2. Since EZH2 represses RKIP transcription, the downregulation of miR-101 indirectly leads to suppression of RKIP, thereby enhancing the metastatic potential of breast cancer cells. Experimental evidence showed that ectopic expression of miR-101 in the metastatic breast cancer cell line MDA-MB-231 resulted in a marked decrease in EZH2 protein levels and a corresponding induction of RKIP protein, supporting the notion that miR-101 acts as a positive regulator of RKIP by targeting and repressing EZH2. This regulatory relationship is specific to miR-101, as overexpression of another microRNA, miR-145, did not alter EZH2 or RKIP levels, underscoring the specificity of the miR-101–EZH2–RKIP axis. Furthermore, HOTAIR, a long noncoding RNA previously implicated in PRC2 recruitment in breast cancer, was shown not to affect RKIP expression, reinforcing the central role of miR-101 in modulating RKIP levels through EZH2 repression in breast cancer. Thus, in breast cancer cells, the dysregulation of miR-101 contributed to RKIP silencing by enabling EZH2 overexpression, which in turn leads to the establishment of repressive chromatin marks on the RKIP promoter, ultimately enhancing cancer invasiveness and metastatic potential [19].

The involvement of both miRNAs and RKIP was also investigated in the context of genes involved in the metastatic processes due to the pivotal role of RKIP of metastasis suppressor. In highly invasive breast cancer cell lines, including MDA-MB-231 and MCF-7, miR-224 expression was significantly upregulated compared to non-invasive or normal breast epithelial cells. This upregulation was associated with increased cell invasiveness, as demonstrated by multiple functional assays including Transwell migration, 3D Matrigel invasion, and wound healing. Experimental overexpression of miR-224 in MDA-MB-231 and MCF-7 cells leads to enhanced invasive capabilities, while its inhibition suppresses invasion. Mechanistically, miR-224 directly binds to the 3′-UTR of RKIP mRNA, resulting in translational repression and reduced RKIP protein levels. This interaction was confirmed by reporter assays in which the activity of RKIP 3′-UTR-linked reporters was suppressed by miR-224 and restored by mutations in the miR-224 binding site or by the use of miR-224 inhibitors. The downregulation of RKIP by miR-224 subsequently derepressed the expression of pro-metastatic genes such as MMP1, OPN, and CXCR4, enhancing the metastatic potential of breast cancer cells. Notably, when RKIP was ectopically overexpressed using a construct lacking the 3′-UTR (and thus resistant to miR-224 targeting), the effects of miR-224 on cell invasion and pro-metastatic gene expression were reversed, highlighting that RKIP is a key functional target mediating the pro-metastatic role of miR-224. These findings collectively support the role of miR-224 as an oncomiR in breast cancer, where its upregulation promotes metastasis by suppressing the RKIP metastasis suppressor pathway. Understanding this miRNA-mediated mechanism provides insight into the molecular basis of breast cancer progression and points to miR-224 and RKIP as potential therapeutic targets [105].

The complex interaction among miRNAs, RKIP and HMGA2 was also investigated by the groups of Sun M (2014) and Zou Q (2016). Sun and colleagues demonstrated that RKIP suppresses metastatic progression by modulating the expression of the downstream target HMGA2, which in turn positively influences the expression of the anti-metastatic miR-200b. They also demonstrated that high miR-200b levels directly repressed the expression of LOX (lysyl oxidase), a gene critical for extracellular matrix remodeling and invasion. Specifically, miR-200b binds to a conserved site in the 3′UTR of LOX mRNA, resulting in decreased LOX protein levels and subsequently reduced cell invasiveness. Furthermore, RKIP indirectly inhibits another pro-metastatic gene, SDC2, through the same HMGA2-dependent pathway. However, unlike LOX, SDC2 is not a direct target of miR-200b. Instead, its expression was regulated by HMGA2 via a miR-200b-independent mechanism. When

RKIP is expressed, HMGA2 levels decrease, leading to a consequent reduction in SDC2 expression. This was validated by experiments showing that HMGA2 knockdown or RKIP overexpression reduces SDC2 mRNA and protein levels in various aggressive breast cancer cell lines. While miR-200b transfection significantly suppresses LOX and reduces invasiveness, it does not affect SDC2 levels, confirming that SDC2 is not under direct control of miR-200b. Overall, the results obtained confirmed that RKIP is a master regulator of two distinct but converging anti-metastatic pathways: one miR-200b-dependent pathway that inhibits LOX, and one miR-200b-independent pathway that suppresses SDC2 through HMGA2 repression; the RKIP/HMGA2/miR-200b/LOX axis and the RKIP/HMGA2/SDC2 axis [106].

The study by Zou Q and collaborators further highlighted the regulatory role of both RKIP and HMGA2 in breast cancer and their interaction with miRNAs. Specifically, they identified a clear inverse relationship between RKIP expression and the progression of breast cancer, demonstrating that both RKIP and miR-185 were significantly downregulated in breast cancer tissues and cell lines compared to normal counterparts. These data suggested that RKIP positively regulates the expression of miR-185, and experimental knockdown of RKIP resulted in decreased levels of miR-185, establishing a direct link between these two factors. Functionally, overexpression of RKIP leads to inhibition of breast cancer cell proliferation and invasion, effects that are mediated, in part, through upregulation of miR-185. Notably, miR-185 acts as a tumor suppressor by directly targeting and suppressing HMGA2, known to be associated with tumorigenesis, invasion, and poor prognosis. In cell-based assays, miR-185 overexpression significantly reduced both growth and invasion of breast cancer cells, and this inhibitory effect was reversed by the overexpression of HMGA2, confirming that HMGA2 is a functional downstream target of miR-185. Moreover, luciferase reporter assays confirm that miR-185 binds directly to the 3'UTR of HMGA2 mRNA, downregulating its expression. Notably, when RKIP is silenced, the suppressive effects of miR-185 on HMGA2 and cancer cell invasion are attenuated, further reinforcing the regulatory cascade where RKIP induces miR-185, which in turn represses HMGA2, thereby limiting cancer aggressiveness. The findings suggest that the anti-metastatic role of RKIP in breast cancer is not exerted solely through direct signaling inhibition (such as through the MAPK pathway), but also through epigenetic regulation via microRNAs like miR-185. This RKIP/miR-185/HMGA2 axis offers mechanistic insight into the suppression of breast cancer invasion and metastasis that could serve as a biomolecular signature for identifying high-risk breast cancers and as a potential therapeutic target [107].

Finally, Labbozzetta M et al. (2015) investigated the role of RKIP and miRNAs in triple-negative breast cancer cells. First, the authors confirmed the low expression levels of RKIP in SUM 159 cells both at the mRNA and protein levels. By treating cells with a demethylating agent and the histone deacetylase inhibitor trichostatin A, the authors observed that promoter hypermethylation and histone deacetylation, along with activation of the transcription factor NF-κB, significantly contribute to RKIP suppression. The authors also investigated the molecular interaction between miR-224 and RKIP; however, miR-224 was found to have no effect on RKIP expression in SUM 159 cells. This was demonstrated by transfection experiments using both precursor and inhibitor forms of miR-224, which failed to modulate RKIP mRNA or protein levels, and by correlation analyses across various breast cancer cell lines that revealed no significant inverse relationship between miR-224 and RKIP expression. These findings underscore the context-dependent nature of RKIP regulation in breast cancer and suggest that miR-224 does not universally govern RKIP suppression across TNBC models.

Although contrasting with the observations reported by Huang et al., the results obtained by Labbozzetta and colleagues require further validation. Indeed, following miR-224 overexpression and silencing in SUM159 cells, the authors did not report the functional effects of these experiments on RKIP expression levels. Furthermore, the MDA-MB-231 cells used by Huang represent metastatic TNBC cells, whereas the SUM159 cells used by Labbozzetta are primary TNBC cells. Therefore, since RKIP modulation is more widely reported in metastatic settings, the effect of miR-224 on RKIP expression may also depend on the tumor context in which it is investigated [94,105]. In addition, Poma P and colleagues, in other tumor models, postulated a decoy role for the pseudogene PEBP1P1, which may sequester miR-224 and thereby relieve its suppressive effect on RKIP [95]. Overall, the results obtained highlighted the dominant role of epigenetic alterations and NF-κB signaling in downregulating RKIP in SUM 159 cells, thereby identifying potential therapeutic targets aimed at restoring RKIP function in aggressive breast cancer subtypes [94].

3.2. Prostate cancer

In prostate cancer, RKIP functions as a critical metastasis suppressor. Its antimetastatic role is underscored by its inverse correlation with tumor aggressiveness. Indeed, RKIP expression is markedly reduced or nearly lost in metastatic prostate tumors, which correlates with poor prognosis [123]. However, the molecular mechanisms driving its downregulation during cancer progression were not fully elucidated.

As regards the epigenetic regulation of RKIP in prostate cancer, three different studies have highlighted the involvement of miRNAs in both direct and indirect regulatory mechanisms of RKIP.

Specifically, besides their findings obtained on breast cancer, Ren G and colleagues (2012) revealed that RKIP expression was transcriptionally repressed by the overexpression of EZH2 also in aggressive prostate cancer and contributes to tumor progression and metastasis by silencing tumor suppressor genes. More in detail, by using prostate cancer cell models, they demonstrated that genetic manipulation of EZH2 expression directly influences RKIP mRNA and protein levels. In low EZH2-expressing cells (such as LNCaP), overexpression of EZH2 significantly decreases RKIP expression, whereas silencing EZH2 in high EZH2-expressing metastatic DU145 cells restores RKIP expression. This suggests that RKIP derepression can be induced through EZH2 inhibition, highlighting a key epigenetic mechanism controlling prostate cancer metastasis. They also demonstrated that this axis is regulated by miR-101, which directly represses EZH2 (Ren G et al., 2012).

Indeed, miR-101 was found downregulated in prostate cancer cell lines and tissues, correlating with increased EZH2 levels and consequent RKIP repression. From a functional point of view, they demonstrated that the ectopic expression of miR-101 restores RKIP levels by suppressing EZH2, establishing miR-101 as a significant modulator of the EZH2-RKIP pathway and a potential therapeutic target to hinder metastatic progression [19].

Moreover, the repression of RKIP is not solely mediated by EZH2-dependent H3K27 trimethylation but also involves other epigenetic modifications and repressive enzymes like histone deacetylases (HDACs). Treatment with HDAC inhibitors such as SAHA abrogates EZH2's suppressive effect on RKIP, indicating a cooperative mechanism of chromatin remodeling and transcriptional silencing at the RKIP promoter. Collectively, these findings suggest that during prostate cancer progression, epigenetic deregulation caused by EZH2 overexpression and miR-101 downregulation results in RKIP transcriptional repression, facilitating metastasis. This molecular axis offers promising avenues for targeted therapies aimed at restoring RKIP expression to counteract tumor aggressiveness [19].

Similarly, Du Y and colleagues (2017) investigated the functional interplay between miR-543 and RKIP in prostate cancer progression, using both clinical specimens and established prostate cancer cell line models. In particular, they analyzed 42 prostate cancer patient samples and identified a strong inverse correlation between miR-543 and RKIP expression levels. Specifically, metastatic prostate cancer tissues exhibited significantly elevated miR-543 expression and concurrently reduced RKIP levels compared to non-metastatic tissues. This observation suggested a potential regulatory relationship where miR-543 may

suppress RKIP, a known tumor suppressor protein. To demonstrate the direct association between miR-543 and RKIP, they also evaluated the expression levels of both factors in LNCaP (non-metastatic) and C4—2B (metastatic) cell lines. Consistent with the clinical data, C4—2B cells showed higher miR-543 and lower RKIP expression relative to LNCaP cells.

Functional experiments manipulating miR-543 levels further clarified its role in prostate cancer pathogenesis, revealing that its overexpression in LNCaP cells reduced RKIP expression and enhanced cell proliferation, invasion, and EMT markers (with the reduction of E-cadherin and the increment of vimentin). Conversely, inhibition of miR-543 in C4—2B cells restored RKIP levels and suppressed proliferation and EMT traits. Furthermore, luciferase reporter assay confirmed the direct link between miR-543 and RKIP. The in vitro findings were also confirmed in vivo, where they also performed orthotopic implantation of LNCaP cells stably overexpressing miR-543 into nude mice, demonstrating the formation of significantly larger tumors with enhanced EMT features.

Finally, by administering a miR-543 antagomir to C4—2B cells injected in mice, they observed a suppression of tumor growth and EMT progression, reinforcing the functional importance of miR-543-RKIP regulation in prostate cancer metastasis [108].

The third report on the miRNA modulation of RKIP in prostate cancer was another study by the same research group of Du Y and colleagues. In this other study, the researchers explored the molecular mechanisms driving prostate cancer progression, with a particular focus on the interplay among the lncRNA XIST, miR-23a, and RKIP. Using a range of in vitro and in vivo prostate cancer models, including cell lines (DU145, LNCaP, and PC3) and xenograft mouse models, Du demonstrated a ceRNA-based regulatory pathway in which XIST functions as a tumor suppressor. Firstly, they observed a significant upregulation of miR-23a in DU145, LNCaP and PC3 cells compared to the non-cancerous prostate epithelial line RWPE1. Moreover, patient samples revealed that miR-23a expression was higher in metastatic prostate tumors than in non-metastatic and adjacent normal tissues. Importantly, a negative correlation between miR-23a and XIST expression was observed in a cohort of 62 prostate cancer patients. In functional in vitro experiments, they also demonstrated that miR-23a did not affect XIST levels, while forced expression of XIST led to a significant downregulation of miR-23a, suggesting a unidirectional regulatory relationship further demonstrating that XIST directly binds to miR-23a, functioning as a molecular sponge as demonstrated by an Ago2-RIP assay. In this complex ceRNA scenario, miR-23a was predicted to target the 3′UTR of RKIP mRNA in silico, a hypothesis confirmed by luciferase reporter assays. Western blotting further showed that miR-23a downregulated RKIP protein expression, while inhibition of miR-23a led to its upregulation, establishing a direct and functional miRNA-RKIP axis.

To determine whether XIST regulates RKIP via miR-23a, the authors finally conducted rescue experiments where the overexpression of XIST led to a marked increase in RKIP levels, but this effect was abrogated by co-transfection with miR-23a mimics, confirming that XIST-mediated upregulation of RKIP is miR-23a-dependent. Conversely, silencing of XIST reduced RKIP expression, but this reduction was rescued by inhibiting miR-23a. Also in mice, tumors derived from XIST-overexpressing cells grew significantly slower, with smaller volumes and weights compared to controls. Immunohistochemical analysis confirmed reduced proliferation (fewer Ki67-positive cells) and increased RKIP expression in XIST-overexpressing tumors, reinforcing the suppressive role of XIST in prostate tumorigenesis through modulation of the miR-23a–RKIP axis [109].

3.3. Lung cancer

RKIP functions as a key tumor suppressor in lung cancer, particularly in non-small cell lung cancer (NSCLC). It negatively regulates both the Raf-1/MEK/ERK signaling cascade and the NF-κB pathway [124].

Several studies have demonstrated RKIP downregulation in lung cancer and its association with enhanced EMT, increased invasion and metastatic potential and a poor overall prognosis and resistance to chemotherapy and targeted therapies [125,126].

Multiple mechanisms are involved in RKIP suppression, including RKIP miRNA-mediating targeting responsible for its repression and consequently lung cancer aggressiveness.

The first record describing miRNA involvement in RKIP regulation dates back 2014 when Li J and colleagues explored the molecular mechanisms behind cisplatin resistance and metastatic progression in NSCLC, focusing on the regulatory axis involving miR-27a and its downstream target RKIP. The authors utilized cisplatin-sensitive and -resistant A549 cells, observing a 5.6-fold increment of miR-27a in resistant cells. Functional studies using miR-27a mimics and inhibitors demonstrated that overexpression of miR-27a in A549 cells induced EMT-like phenotypes and cisplatin resistance, whereas inhibition of miR-27a in resistant clones reversed these effects. These findings were validated in additional lung cancer lines (H1299 and H1395), confirming the effects of miR-27a in drug resistance [110].

Through further bioinformatics analyses, the authors identified a binding site for miR-27a in the 3′UTR of RKIP mRNA and then validated this interaction through luciferase reporter assays, demonstrating that miR-27a negatively regulates RKIP expression via direct binding to its 3′UTR [110].

Li and collaborators also silenced RKIP in A549 cells, demonstrating enhanced EMT features and decreased sensitivity to cisplatin. Conversely, reintroducing RKIP (lacking the 3′UTR and thus resistant to miR-27a suppression) partially reversed the EMT phenotype and drug resistance, even in the presence of elevated miR-27a. Such in vitro findings were also confirmed in mice and in clinical samples obtained from NSCLC patients demonstrating, respectively, that A549 cells overexpressing miR-27a formed significantly more lung metastases and were less responsive to cisplatin and that patients with high miR-27a expression showed low RKIP levels, poor response to cisplatin therapy, and shorter overall survival confirming the negative correlation existing between miR-27a and RKIP mRNA levels ($r = -0.691$, $P < 0.01$) [110].

Another study demonstrated an indirect role of miRNAs in the regulation of RKIP. In particular, Li H and colleagues (2016), demonstrated that miR-150 functions as a pro-metastatic microRNA in NSCLC primarily by directly targeting and suppressing FOXO4, a tumor suppressor transcription factor. The researchers observed that miR-150 expression is significantly upregulated in NSCLC tissues, particularly in metastatic samples, while FOXO4 levels are markedly reduced, indicating an inverse correlation between these two factors. Functionally, overexpression of miR-150 enhances cellular migration and induces EMT, whereas inhibition of miR-150 suppresses these processes in A549 and H460 NSCLC cells. Similarly, FOXO4 knockdown mimics the effects of miR-150 overexpression, supporting the conclusion that FOXO4 is a direct and functionally critical downstream target of miR-150 via its 3′UTR. Importantly, the loss of FOXO4 leads to the activation of the NF-κB/Snail signaling axis, which promotes EMT through repression of E-cadherin and upregulation of mesenchymal markers such as vimentin and N-cadherin. Since RKIP is known to be a downstream effector that is negatively regulated by NF-κB and Snail, the authors suggested that miR-150 affects RKIP expression **i**ndirectly. Specifically, miR-150 suppresses FOXO4, which normally inhibits NF-κB activity; in absence of FOXO4, NF-κB becomes activated, leading to increased Snail expression, which in turn represses RKIP transcription. Therefore, the downregulation of RKIP is not due to direct interaction with miR-150 but is mediated through the FOXO4–NF-κB–Snail axis. Overall, the authors' findings suggested that FOXO4 is a key mediator of miR-150-induced metastatic behavior in NSCLC. The dysregulation of the miR-150–FOXO4 axis promotes EMT and metastatic potential by indirectly repressing RKIP through activation of NF-κB/Snail signaling. This regulatory cascade presents a promising therapeutic target for limiting metastasis in NSCLC [111].

As described for prostate cancer, miR-543 seems to be involved also in RKIP suppression in lung cancer. Indeed, Gong Z et al. (2020) investigated the tumor-suppressive role of the GATA6-AS1/miR-543 axis and its role in the regulation of RKIP. In particular, they found a significant reduced RKIP expression in NSCLC tissues and cell lines, suggesting that its downregulation may be a key feature of tumor progression. This reduction seems to be associated with the concomitant decrement of GATA6-AS1 levels observed in patients with larger tumor sizes and increased lymph node metastasis, indicating a possible prognostic value of both factors. The authors further demonstrated in vitro (H1299, H460, A549) that GATA6-AS1 overexpression inhibits the proliferation, migration, invasion, and EMT of tumor cells, while its knockdown leads to opposite effects. By performing dual-luciferase reporter assays, the authors demonstrated that GATA6-AS1 exerts its tumor-suppressive function by sponging miR-543 that in turn is a direct downstream target of miR-543. The inverse correlation between miR-543 and RKIP, as well as the positive correlation between GATA6-AS1 and RKIP, was validated in NSCLC tissues, supporting the pivotal role of the GATA6-AS1/miR-543/RKIP axis in modulating NSCLC malignancy through the modulation of the STAT3 signaling pathway [112].

Finally, Lin Z and collaborators (2022) recently demonstrated the involvement of miR-3940-5p in lung adenocarcinoma using both bioinformatic analyses and in vitro experiments. They found that miR-3940-5p is significantly overexpressed in lung cancer tissues and cell lines, and its high expression correlates with advanced tumor stage and poor clinical outcomes, including shorter overall, disease-specific, and progression-free survival (Lin Z et al., 2022).

Through target prediction analyses, they identified several potential target genes, including RKIP. Although direct regulation was not experimentally confirmed, the study observed an inverse correlation between miR-3940-5p and RKIP expression, suggesting that miR-3940-5p may contribute to tumor progression by downregulating RKIP, thereby allowing the activation of oncogenic pathways [113].

Functional enrichment analyses showed that the miRNA's targets are involved in cancer-related pathways such as PI3K-Akt and p53 signaling. Additionally, immune cell infiltration analysis indicated that miR-3940-5p may alter the tumor immune microenvironment, potentially promoting immune evasion. Taken together, these findings highlighted that miR-3940-5p may play an oncogenic role in lung cancer, possibly by suppressing tumor suppressors like RKIP and modulating both intracellular signaling and immune responses, making it a potential prognostic marker and therapeutic target [113].

In summary, these four studies confirm the involvement of multiple miRNAs in the downregulation of RKIP through both direct and indirect mechanisms. Indeed, miR-27a, miR-543, and miR-3940-5p directly target and suppress RKIP, while miR-150 indirectly downregulates RKIP through the FOXO4–NF-κB–Snail signaling axis, collectively contributing to lung cancer progression by disrupting RKIP-mediated tumor-suppressive pathways and promoting cancer progression, metastasis, and treatment resistance.

3.4. Gastric cancer

Multiple studies have consistently shown that RKIP expression is significantly reduced in gastric cancer tissues and cell lines compared to normal gastric mucosa. This downregulation correlates with advanced tumor stage, deeper invasion, lymph node metastasis, and worse patient prognosis. Functional experiments demonstrated that overexpression of RKIP in gastric cancer cells leads to reduced proliferation, invasion, metastasis, and increased apoptosis, confirming its tumor-suppressive role [127–129].

These findings suggest that upstream regulatory mechanisms, potentially involving microRNAs, may play a significant role in modulating RKIP expression and thus represent promising targets for therapeutic intervention.

In 2014, Liu H and colleagues explored the expression and function of RKIP in gastric cancer cell lines (GC-7901, MGC80–3, and MKN45) in comparison with normal gastric mucosal epithelial cells (GES-1). Using qRT-PCR and Western blot analysis, they demonstrated that RKIP expression was significantly downregulated in all gastric cancer cell lines analyzed and that this downregulation was associated with apoptosis blocking and cell migration supporting the role of RKIP as a negative regulator of tumorigenic properties in gastric cancer cells. Then the authors shifted their attention to the regulatory role of microRNAs, with a specific focus on miR-224. Through bioinformatic analyses, they identified miR-224 as a direct regulator of RKIP, confirming the interaction between these two factors through luciferase reporter assay. Subsequent experiments revealed that overexpression of miR-224 in SGC-7901 cells resulted in a marked reduction of RKIP protein levels, as detected by Western blotting. Functionally, this was associated with enhanced cell viability, proliferation, and migration, alongside reduced apoptosis, mimicking the inverse of RKIP overexpression effects. Interestingly, co-transfection of RKIP and miR-224 mimic neutralized these changes, suggesting that miR-224 exerts its oncogenic effects in part through the downregulation of RKIP. Overall, these findings suggest that therapeutic strategies aimed at enhancing RKIP expression or inhibiting miR-224 activity may offer new avenues for the treatment of gastric cancer [114].

Another study investigated the role of two different miRNAs in the regulation of RKIP and in the progression of gastric cancer. Li Y and colleagues (2020) focused their attention on the epigenetic regulator Bmi-1 and its downstream effectors, including RKIP, miR-27a and miR-155. The researchers demonstrated that Bmi-1 represses RKIP post-transcriptionally, as Bmi-1 overexpression reduced RKIP protein levels in non-tumorigenic GES-1 gastric cells without affecting its mRNA or protein degradation. miRNA microarray and bioinformatic analysis (TargetScan) identified miR-27a and miR-155 as Bmi-1-induced microRNAs that directly target RKIP.

Functional assays in BGC823 and SGC7901 GC cell lines showed that Bmi-1 knockdown reduced cell proliferation, migration, invasion, and colony formation. This phenotype was reversed by co-expression of miR-27a or miR-155 mimics. Additionally, Bmi-1 depletion enhanced sensitivity to 5-fluorouracil and oxaliplatin, increasing apoptosis. Again, miR-27a and miR-155 mimics reversed this chemosensitization by suppressing RKIP. In vivo, xenograft models in nude mice confirmed that Bmi-1 knockdown suppressed tumor growth and metastasis to lungs and liver, while miR-27a or miR-155 overexpression restored tumor aggressiveness and conferred resistance to 5-fluorouracil. Immunohistochemistry and Western blotting of tumor samples supported a negative correlation between Bmi-1 and RKIP expression, alongside altered levels of metastasis and apoptosis-related proteins (Vimentin, E-cadherin, Bax, Bcl-2). Overall, these findings suggested that the Bmi-1/miR-27a/miR-155 axis is responsible for the regulation of GC metastasis and chemoresistance through RKIP repression. Clinically, the distinct expression patterns of miR-27a and miR-155 across GC histological subtypes suggest their potential as biomarkers or therapeutic targets [115].

Finally, Hu K et al. (2020) explored novel molecular mechanisms involved in GC progression, particularly focusing on the tumor-suppressive role of the circular RNA circMTO1 and its interaction with microRNAs and RKIP. circMTO1 expression was significantly downregulated in GC tissues and cell lines compared to normal controls. Functional assays revealed that overexpression of circMTO1 inhibited GC cell proliferation, migration, invasion, and EMT, supporting a tumor-suppressive role for circMTO1 in GC. Through bioinformatics analysis (starBase v2.0), RNA pull-down, and luciferase reporter assays, the authors identified miR-3200-5p as a direct target of circMTO1. Notably, miR-3200-5p expression was upregulated in GC tissues and cell lines, and its levels were inversely correlated with circMTO1 expression, suggesting a negative regulatory relationship. Further investigation uncovered that RKIP was a direct downstream target of miR-3200-5p and was significantly downregulated in GC tissues. In contrast, circMTO1 overexpression led to increased RKIP levels, while miR-3200-

5p mimics suppressed it. These results collectively suggested a circMTO1/miR-3200-5p/RKIP axis in the regulation of GC. In summary, this study uncovers a novel regulatory pathway in gastric cancer whereby circMTO1 acts as a tumor suppressor by sponging miR-3200-5p, thereby releasing its inhibitory effect on RKIP/PEBP1 [116].

In conclusion, these studies collectively highlight RKIP as a critical tumor suppressor in gastric cancer, whose downregulation is consistently linked to tumor progression, metastasis, and poor prognosis. A recurring theme across multiple models is the post-transcriptional repression of RKIP by specific oncogenic microRNAs, including miR-224, miR-27a, miR-155, and miR-3200-5p. These miRNAs act either independently or as part of broader regulatory networks involving oncogenes like Bmi-1 or tumor-suppressive circRNAs like circMTO1, establishing complex feedback loops that finely tune RKIP expression. Therapeutically, targeting these upstream regulators, particularly through miRNA inhibition or circRNA restoration, offers promising strategies to restore RKIP function and suppress gastric cancer aggressiveness and chemoresistance.

3.5. Liver cancer

RKIP plays a key role in the suppression of tumorigenesis by negatively regulating several signaling in hepatocellular carcinoma (HCC). In HCC, RKIP expression is markedly downregulated and this loss is associated with enhanced tumor cell proliferation, migration, and metastasis. Mechanistic studies have demonstrated that restoring RKIP levels can inhibit the activation of oncogenic pathways like ERK/MAPK and IGF—I, thereby suppressing tumor growth both in vitro and in vivo [130,131]. Moreover, beyond its role in cancer, reduced RKIP expression has also been implicated in exacerbating chronic liver injury and hepatic fibrosis by promoting hepatic stellate cell activation and collagen deposition [132].

Altogether, RKIP emerges as a critical suppressor of liver disease progression, with its downregulation contributing to both fibrosis and liver cancer pathogenesis. Given its pivotal role in liver disease and cancer progression, understanding the mechanisms underlying RKIP downregulation is crucial. Among these, miRNA-mediated regulation has emerged as a significant post-transcriptional mechanism controlling RKIP expression in HCC. In particular, Poma P and colleagues investigated the epigenetic regulation of RKIP in HCC by investigating both promoter methylation and miRNAs. While promoter methylation shown to play a minor role (only observed in Hep3B cells) in RKIP regulation, the main focus was on post-transcriptional regulation, particularly mediated by miR-224. As described above, miR-224 is known to affect proliferation, apoptosis, and invasion of cancer cells and can directly suppress RKIP in other tumors. To support the regulating role of miR-224 also in HCC, the authors compared miR-224 expression across different HCC cell lines, finding that HA22T/VGH cells express high levels of miR-224, HepG2 shows low expression, and Hep3B has none. Surprisingly, when miR-224 was inhibited in HA22T/VGH cells using an anti-miR inhibitor, no change in RKIP mRNA or protein levels was observed, even after 72 h. Similarly, introducing a miR-224 precursor into Hep3B cells failed to reduce RKIP levels. These findings clearly suggest that miR-224 does not regulate RKIP expression in the HCC cell lines examined. The authors hypothesize that this lack of effect might be due to the action of PEBP1P1, a pseudogene of RKIP that can act as “miRNA decoys" sequestering miRNAs and preventing them from binding to their actual targets, highlighting the complex regulating dynamics of RKIP mediated by miRNAs [95].

Another study investigated the oncogenic role of miR-27a and RKIP in liver cancer proliferation, migration, and resistance to cisplatin (CDDP). First, the authors demonstrated that miR-27a and miR-664b serum levels were found significantly upregulated in liver cancer patients compared to controls, while miR-30a was downregulated. Notably, elevated miR-27a levels were positively correlated with advanced tumor features, including higher TNM staging, multiple tumor nodules, larger tumor size, and vascular invasion, indicating its association with tumor aggressiveness.

To explore the functional implications of miR-27a in liver cancer and RKIP expression, MCC-7721 liver cancer cells were transfected with miR-27a mimics. Post-transfection, these cells showed significantly enhanced proliferation at 48 and 72 h, highlighting the pro-proliferative effect of miR-27a. Additionally, in A549/CDDP cells, a cisplatin-resistant cell line derived from parental A549 cells, miR-27a was found to be upregulated. Functional experiments demonstrated that miR-27a promotes EMT by downregulating E-cadherin and upregulating vimentin, thereby enhancing both cell migration and drug resistance. Such effects were mediated by RKIP, which was a direct downstream target of miR-27a. This was confirmed through in silico prediction and validated by a luciferase reporter assay. The suppression of RKIP was shown to mimic the oncogenic effects of miR-27a, while overexpression of RKIP counteracted the proliferative and cisplatin-resistant phenotype induced by miR-27a. These findings support a model in which miR-27a directly binds to the 3’-UTR of RKIP mRNA, thereby downregulating its expression. Finally, liver cancer tissues obtained from cisplatin non-sensitive patients exhibited significantly higher miR-27a levels and correspondingly lower RKIP expression with a negative correlation statistically confirmed [117].

In a third study on the role of miRNA and RKIP in liver cancer, the authors describe how the tumor promoter 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) induces a dual phenotypic response in liver cancer cells, cell cycle arrest and enhanced migration, via a signaling cascade involving PKCδ, mitochondrial ROS (mtROS), HSP60, MAPK, and AP-1.

In this intricate pathway, the authors investigated the role of RKIP and a novel microRNA, miR-6134, both playing pivotal roles in modulating the outcome of TPA stimulation. In this context, RKIP was shown to associate with HSP60, a mitochondrial chaperone, thus restraining MAPK activity. Upon TPA stimulation, the activation of PKCδ leads to an increase in mtROS, which oxidizes HSP60 and reduces its affinity for RKIP. This oxidative dissociation occurs both in mitochondria and the cytosol, releasing RKIP’s inhibitory influence and allowing for MAPK activation. This is significant because the release of RKIP not only lifts the suppression on MAPK-mediated signaling but also enhances HSP60’s ability to act as a signaling mediator, thereby supporting both cell migration and cell cycle arrest depending on the downstream context. In parallel to this protein-level regulatory mechanism, the study uncovers a novel microRNA, miR-6134, which is transcriptionally upregulated as part of the same PKCδ-mtROS-HSP60-MAPK-AP-1 pathway. While AP-1 (composed of c-jun and c-fos) directly activates genes involved in EMT, migration, and invasion, it also indirectly promotes cell cycle arrest by upregulating miR-6134. This miRNA targets CCNE1, a key cyclin essential for G1/S transition, thereby contributing to cell cycle inhibition. Importantly, the promoter of the gene encoding miR-6134 contains a functional AP-1 binding site, indicating that its expression is directly regulated by the AP-1 transcriptional machinery. The identification of miR-6134 adds a new layer of post-transcriptional regulation within this signaling framework, demonstrating how transcription factor networks and miRNA-mediated gene silencing collaborate to enforce complex phenotypic programs in cancer. By mediating repression of CCNE1, miR-6134 helps implement the cell cycle arrest arm of the dichotomous phenotype, complementing AP-1’s role in enhancing invasiveness through direct transcriptional activation of migration-related genes. Together, these findings highlight the dual but coordinated roles of RKIP and miR-6134 in modulating liver cancer progression. RKIP operates as a redox-sensitive gatekeeper of MAPK activity, while miR-6134, under the transcriptional control of AP-1, reinforces cell cycle arrest by targeting key regulators of proliferation. This integrated mechanism suggests that targeting the RKIP-HSP60 interaction or modulating miR-6134 expression could represent novel strategies for interfering with tumor progression in HCC [102].

3.6. Other tumors

Several independent studies have also demonstrated the involvement of miRNAs in the regulation of RKIP in both solid tumors and onco-hematological malignancies.

In 2013, Chen Z and colleagues published an article demonstrating the co-operation between RKIP and miR-98 in suppressing HMGA2, a known pro-metastatic gene responsible for glioma invasiveness. In particular, the findings reveal that RKIP plays an indirect but functionally critical role in regulating miR-98 levels, which in turn suppresses the expression of HMGA2, a known pro-metastatic gene, thereby reducing glioma invasiveness.

In both glioma patient tissues and cell lines, miR-98 and RKIP were consistently downregulated, while HMGA2 was markedly upregulated when compared to normal brain tissues. A significant positive correlation was found between RKIP and miR-98 expression, and a negative correlation between miR-98 and HMGA2 expression, suggesting a potential regulatory axis.

Mechanistically, RKIP acts upstream of miR-98, potentially through transcriptional regulation mechanisms, thus indirectly controlling miR-98 expression. Specifically, upon overexpression of RKIP in glioma cell lines (U251 and U87), there was a significant increase in the levels of primary and mature miR-98, along with a decrease in HMGA2 protein levels. In addition, the suppression of HMGA2 via miR-98 mimic was associated with a marked inhibition of glioma cell invasion, without affecting cell proliferation. Co-transfection of glioma cells with both RKIP and miR-98 mimics led to even greater repression of HMGA2 and more potent inhibition of invasion, reinforcing the synergistic relationship between RKIP and miR-98 in this context and demonstrating their role as promising tumor suppressor factors [118].

As regards the direct regulation of RKIP mediated by miRNAs in hematological tumors, Hatzl S et al. (2016) investigated the regulatory role of the miR-23a/27a/24–2 cluster in the suppression of RKIP in acute myelogenous leukemia (AML). In particular, through an extensive analysis of over 400 primary AML patient samples, the researchers observed a strong inverse correlation between RKIP expression and miR-23a levels supporting the hypothesis that miR-23a may be directly responsible for repressing RKIP expression.

By overexpressing miR-23a using a miRNA mimic in AML cells, the authors observed a significant reduction of RKIP protein levels and, in turn, an increase in cell proliferation, as determined by BrdU/PI and BrdU/7-AAD cell-cycle assays. This proliferation-enhancing effect was not observed when RKIP was ectopically expressed from a construct lacking its natural 3′ untranslated region (3′UTR) targeted by miR-23a. Moreover, stable knockdown of RKIP using lentiviral shRNA also led to increased cell proliferation, mirroring the effects of miR-23a overexpression and establishing a causal link between miR-23a and RKIP suppression. To further confirm the pathogenetic role of the miR-23a/27a/24–2 cluster the authors investigated 14 other cancer types using The Cancer Genome Atlas (TCGA). Among 4342 primary tumor samples, at least one miRNA from the miR-23a/27a/24–2 cluster inversely correlated with RKIP expression in 10 out of 14 cancer types; of these miRNAs. miR-23a emerged as the predominant regulator affecting RKIP expression in nine of these cancers, suggesting its involvement in multiple tumors [23].

More recently, the regulatory role of miR-181a on RKIP expression was demonstrated in nasopharyngeal carcinoma (NPC). In this study, the authors investigated the role of both miR-181a and RKIP in NPC radioresistance mechanisms. By analyzing the expression levels of miRNAs in radiosensitive and radioresistant CNE2 cells, miR-181a was found to be significantly upregulated in resistant clones. High miR-181a expression levels were also observed in clinical samples obtained from cancer patients when compared to non-neoplastic mucosa. Such an increment correlated also with advanced TNM stages and lymph node metastasis, and negatively with overall survival. By using lentiviral vectors, miR-181a was overexpressed in radiosensitive CNE2 cells demonstrating that miR-181a overexpression enhanced radioresistance in CNE2 cells (with increased cell viability, higher colony formation, and decreased apoptosis). Conversely, inhibition of miR-181a in CNE2-IR cells sensitized them to irradiation.

The authors also observed that the overexpression of miR-181a in CNE2 cells led to decreased RKIP mRNA and protein levels, while inhibition of miR-181a in CNE2-IR cells restored RKIP expression. A dual-luciferase reporter assay confirmed that miR-181a directly binds to the 3′ untranslated region (3'UTR) of RKIP mRNA to suppress its expression and the functional silencing and overexpression of RKIP demonstrated that RKIP mediates the radioresistant effects of miR-181a dysregulation in vitro. Finally, xenograft nude mice injected with CNE2 and CNE2-IR cells overexpressing or not miR-181a and RKIP were irradiated (6 Gy) demonstrating that miR-181a promoted resistance to radiation, an effect reversed by RKIP restoration; while, miR-181a inhibition sensitized tumors, a response diminished when RKIP was knocked down. All these findings suggest that the miR-181a/RKIP axis may be a critical regulatory pathway in determining the radiosensitivity of NPC cells and both factors may represent promising therapeutic targets to overcome radioresistance [24].

Another study focused attention on the same miR-181a and its regulating role on EMT in pancreatic cancer via RKIP targeting. Kang H and colleagues (2020) explored the EMT and stemness potential induced by RKIP and miR-181a in pancreatic cancer. First, they demonstrated that RKIP expression is significantly downregulated in pancreatic cancer tissues compared to adjacent normal tissues, as demonstrated by qPCR and western blot analysis. In PANC-1 cell lines transfected with RKIP-expressing plasmids, they observed increased expression of E-cadherin and decreased levels of mesenchymal markers, including N-cadherin, Vimentin, Snail, TGFB1, and Fibronectin, suggesting a suppression of EMT. Additionally, RKIP overexpression reduces the cell ability to form spheres and inhibits both migration and invasion, reinforcing its role as a metastasis suppressor. As regards miR-181a, it was found to be upregulated in pancreatic cancer tissue and transfection of PANC-1 cells with pre-miR-181a leads to a notable downregulation of RKIP at both the mRNA and protein levels, as confirmed by real-time PCR, western blot, and immunofluorescence assays, and EMT progression. Furthermore, miR-181a overexpression enhanced sphere formation and increased the expression of CSC-associated markers CD44 and Tspan8, while also promoting cell migration and invasion. These findings suggest that miR-181a acts as an oncogenic miRNA by downregulating RKIP and promoting both EMT and CSC-like characteristics in pancreatic cancer cells [119].

Finally, Yang L et al. (2022) investigated the role of the pseudogene-derived lncRNA PEBP1P2 in clear cell renal cell carcinoma (ccRCC) and its regulatory impact on RKIP, as well as its interactions with key microRNAs. They demonstrated that PEBP1P2 is significantly downregulated in ccRCC due to transcriptional repression exerted by overexpressed STAT4. Mechanistically PEBP1P2 plays a dual regulatory role since it stabilizes RKIP/PEBP1 mRNA by preventing its degradation, and it acts as a competitive endogenous RNA (ceRNA) that sponges specific oncogenic miRNAs. As regards this latter mechanism, PEBP1P2 regulates the post-transcriptional expression of the transcription factor KLF13 by functioning as a molecular sponge for miR-296, miR-616, and miR-3194. These miRNAs were upregulated in ccRCC and negatively correlated with patient prognosis. These miRNAs also targeted both PEBP1P2 and KLF13 mRNA directly. PEBP1P2 competes with KLF13 mRNA for binding to these miRNAs, thereby protecting KLF13 expression from miRNA-induced repression. Functionally, silencing PEBP1P2 leads to decreased RKIP and KLF13 levels, increased cell migration and invasion, while overexpression of PEBP1P2 has the opposite effect, suggesting a tumor-suppressive role. Clinical data from TCGA corroborate these findings, showing that high PEBP1P2 and PEBP1 expression correlate with better prognosis. In summary, PEBP1P2 exerts anti-metastatic effects in ccRCC by stabilizing RKIP mRNA and by sequestering oncogenic miRNAs that otherwise suppress KLF13. These findings

highlight a complex post-transcriptional regulatory network of RKIP mediated by both miRNAs and lncRNAs since PEBP1P2 modulates the expression of critical metastasis-associated genes [120].

4. Conclusions

The studies described here showed an important post-transcriptional regulatory role played by miRNAs on RKIP expression and consequently on tumor progression, metastasis, and therapy resistance across multiple cancer types. The evidence presented highlights RKIP as a central node in this intricate regulatory network, where its suppression or restoration significantly impacts cancer cell behavior, including invasion, proliferation, EMT, stemness, and radioresistance.

The analysis of the studies reported in the literature on this topic revealed that, despite tissue-specific variations, the regulatory role of miRNAs on RKIP follows a set of recurring principles (Fig. 4).

In many cancers, direct suppression of RKIP through binding to its 3′UTR by microRNAs such as miR-224, miR-27a, miR-23a, miR-543, and miR-181a leads to enhanced proliferation, epithelial–mesenchymal transition, invasion, and therapy resistance [23,24,110,112,114,117,119]. These findings consistently demonstrate that loss of RKIP expression acts as a central event in promoting tumor aggressiveness, and that restoring RKIP counteracts the oncogenic effects of these miRNAs. Alongside this direct repression, several tumors exhibit indirect regulatory loops in which miRNAs control upstream transcriptional repressors of RKIP. A clear example is the miR-101/EZH2/RKIP axis, described in both breast and prostate cancer, where downregulation of miR-101 enables EZH2 overexpression and subsequent transcriptional silencing of RKIP [19]. Similarly, in non-small cell lung cancer, miR-150 indirectly represses RKIP through the FOXO4/NF-κB/Snail cascade, underscoring the existence of alternative routes by which microRNAs converge on RKIP downregulation [111].

Another layer of complexity emerges from the interaction between RKIP and competing endogenous RNAs. lncRNAs such as XIST and GATA6-AS1, circRNAs such as circMTO1, and pseudogenes like PEBP1P1 or PEBP1P2 have all been shown to sequester oncogenic miRNAs, thereby protecting RKIP from suppression [95,108,112,116,120]. Interestingly, this mechanism provides a plausible explanation for tissue-specific discrepancies, such as the inability of miR-224 to repress RKIP in hepatocellular carcinoma, in contrast to its clear inhibitory role in breast and gastric cancer. These decoy effects highlight the importance of cellular context in determining whether miRNA-mediated repression of RKIP is functionally relevant [95].

It is also noteworthy that RKIP not only serves as a target of miRNAs but can itself modulate the expression of tumor-suppressive miRNAs. In breast cancer, RKIP promotes the expression of let-7 and miR-200b, which in turn inhibit pro-metastatic genes such as HMGA2, Snail, and LOX [106]. This reciprocal regulation establishes RKIP as both a downstream effector and an upstream modulator within miRNA-driven networks, reinforcing its role as a master regulator of metastatic behavior.

While most evidence regarding miRNA RKIP regulation comes from solid tumors, studies in hematological malignancies suggest both parallels and important distinctions. In solid tumors, miRNA-mediated suppression of RKIP predominantly drives epithelial–mesenchymal transition, invasion, and metastatic dissemination. By contrast, in acute myeloid leukemia, miR-23a emerges as the predominant regulator of RKIP, where its loss does not drive EMT or stromal invasion but rather

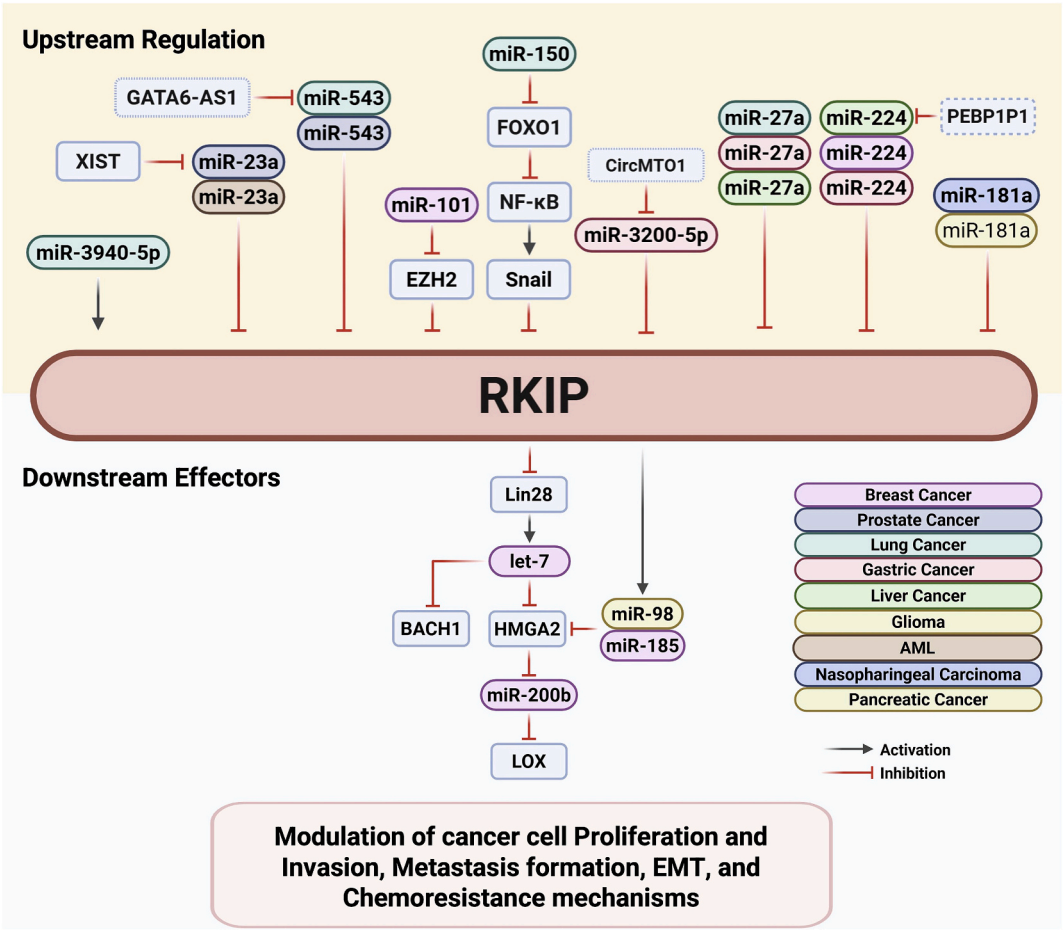

Fig. 4. Schematic representation of the upstream miRNAs regulating RKIP and their downstream effectors according to each cancer type.

enhances proliferation and survival of malignant blasts. The lack of a structural epithelial context means that RKIP suppression in leukemia primarily contributes to unchecked growth and impaired differentiation, rather than invasion and metastasis [23].

Overall, at the signaling level, shared consequences of miRNA-mediated RKIP suppression include the activation of EMT, deregulation of pro-metastatic transcription factors, remodeling of the extracellular matrix, and increased survival signaling. Specifically, decreased RKIP was associated with the activation of STAT3, NF-κB, or MAPK pathways that promote proliferation and resistance to cell death [94,102,111,112]. Collectively, these findings demonstrate that a limited number of miRNAs, recurrently deregulated across cancers, orchestrate a set of conserved oncogenic pathways through suppression of RKIP, establishing a unifying mechanism of metastatic progression.

Building on these insights, targeting the miRNA/RKIP axis offers a promising therapeutic avenue. The use of miRNA mimics to restore tumor-suppressive microRNAs or antagomiRs to inhibit oncogenic miRNAs is gaining great attention in the scientific community [133]. These approaches could effectively restore RKIP expression, thereby counteracting proliferation, invasion, EMT, and therapy resistance across multiple cancer types. However, to the best of our knowledge, no preclinical or clinical studies using RNA-based therapeutics to regulate RKIP are currently ongoing. Among the RKIP-targeting miRNAs, only miR-155 is currently under investigation as a potential therapeutic target in multiple hematological malignancies, including Cutaneous T-cell Lymphoma (CTCL), Chronic Lymphocytic Leukemia (CLL), Diffuse large B-cell Lymphoma (DLBCL), and Adult T-cell leukemia/lymphoma (ATLL) and Mycosis Fungoides (MF). Indeed, clinical trials using Cobomarsen/MRG-106, an LNA anti-miR-155, demonstrated the efficacy of this treatment in alleviating clinical symptoms in a small cohort (n. 6) of CTCL patients and in patients with MF [134–136].

Similarly, modulation of ceRNAs, including lncRNAs or circular RNAs that sequester oncogenic miRNAs, could represent an additional strategy to stabilize RKIP levels. Nevertheless, several challenges remain for clinical translation, including efficient and tumor-specific delivery of RNA-based therapeutics, minimization of off-target effects, and consideration of tumor heterogeneity and context-dependent regulatory networks [133]. Overcoming these barriers will require rigorous preclinical validation, optimization of delivery systems, and careful integration with existing therapies. Despite these challenges, the conserved role of RKIP as a central node in miRNA-mediated oncogenic pathways underscores its potential as a target for precision oncology interventions.

Future studies should prioritize the clinical validation of these regulatory pathways, evaluate their potential as predictive biomarkers, and explore combinatorial approaches integrating RNA-based therapies with conventional treatments. Additionally, expanding research into the dynamics of RKIP regulation in the tumor microenvironment and resistance mechanisms will be essential to translate these findings into effective precision oncology interventions.

Funding

This work was supported in part by: European Union—NextGenerationEU through the Italian Ministry of University and Research under PNRR M4C2—Action 1.4—Call “Potenziamento strutture di ricerca e creazione di campioni nazionali di R&S”—Project “National Center for Gene Therapy and Drugs based on RNA Technology” (CN00000041) to Prof. Massimo Libra (CUP: E63C22000950006) and by the PIAno di inCEntivi per la RIcerca di Ateneo 2024/2026 (C.d.A. del 10.05.2024)—Linea di Intervento 1 “Progetti di ricerca collaborativa”, University of Catania (Catania, Italy)—Project Code: BioEpiRes to Prof. Luca Falzone.

Declaration of competing interest

Massimo Libra is guest editor of the Special Issue “Prognostic and Therapeutic Implications of RKIP and YY1 in Cancer and Inflammatory Diseases" but he had no personal involvement in the reviewing process or any influence in terms of adjudicating on the final decision for this article.

Data availability

No data was used for the research described in the article.

References

[1] L.H. Wang, C.F. Wu, N. Rajasekaran, Y.K. Shin, Loss of tumor suppressor gene function in human cancer: an overview, Cell. Physiol. Biochem. 51 (2018) 2647–2693, https://doi.org/10.1159/000495956.
[2] T.C. Dakal, B. Dhabhai, A. Pant, K. Moar, K. Chaudhary, V. Yadav, V. Ranga, N. K. Sharma, A. Kumar, P.K. Maurya, J. Maciaczyk, I.G.H. Schmidt-Wolf, A. Sharma, Oncogenes and tumor suppressor genes: functions and roles in cancers, MedComm 5 (2024) (2020) e582, https://doi.org/10.1002/mco2.582.
[3] J. Escara-Wilke, K. Yeung, E.T. Keller, Raf kinase inhibitor protein (RKIP) in cancer, Cancer Metastasis Rev. 31 (2012) 615–620, https://doi.org/10.1007/s10555-012-9365-9.
[4] K. Yeung, T. Seitz, S. Li, P. Janosch, B. McFerran, C. Kaiser, F. Fee, K. D. Katsanakis, D.W. Rose, H. Mischak, J.M. Sedivy, W. Kolch, Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP, Nature 401 (1999) 173–177, https://doi.org/10.1038/43686.
[5] K. Lorenz, M.J. Lohse, U. Quitterer, Protein kinase C switches the Raf kinase inhibitor from Raf-1 to GRK-2, Nature 426 (2003) 574–579, https://doi.org/10.1038/nature02158.
[6] K.C. Yeung, D.W. Rose, A.S. Dhillon, D. Yaros, M. Gustafsson, D. Chatterjee, B. McFerran, J. Wyche, W. Kolch, J.M. Sedivy, Raf kinase inhibitor protein interacts with NF-kappaB-inducing kinase and TAK1 and inhibits NF-kappaB activation, Mol. Cell. Biol. 21 (2001) 7207–7217, https://doi.org/10.1128/MCB.21.21.7207-7217.2001.
[7] X.M. Zhang, C. Zhou, H. Gu, L. Yan, G.Y. Zhang, Correlation of RKIP, STAT3 and cyclin D1 expression in pathogenesis of gastric cancer, Int. J. Clin. Exp. Pathol. 7 (2014) 5902–5908.
[8] Q.Y. He, H.M. Yi, H. Yi, T. Xiao, J.Q. Qu, L. Yuan, J.F. Zhu, J.Y. Li, Y.Y. Wang, L. N. Li, J. Feng, S.S. Lu, Z.Q. Xiao, Reduction of RKIP expression promotes nasopharyngeal carcinoma invasion and metastasis by activating Stat3 signaling, Oncotarget 6 (2015) 16422–16436, https://doi.org/10.18632/oncotarget.3847.
[9] F. Al-Mulla, M.S. Bitar, M. Al-Maghrebi, A.I. Behbehani, W. Al-Ali, O. Rath, B. Doyle, K.Y. Tan, A. Pitt, W. Kolch, Raf kinase inhibitor protein RKIP enhances signaling by glycogen synthase kinase-3β, Cancer Res. 71 (2011) 1334–1343, https://doi.org/10.1158/0008-5472.CAN-10-3102.
[10] M. Moghaddam, S. Vivarelli, L. Falzone, M. Libra, B. Bonavida, Cancer resistance via the downregulation of the tumor suppressors *RKIP* and *PTEN* expressions: therapeutic implications, Explor. Target. Antitumor. Ther. 4 (2023) 170–207, https://doi.org/10.37349/etat.2023.00128.
[11] F. Al-Mulla, M.S. Bitar, J.P. Thiery, T.T. Zea, D. Chatterjee, L. Bennett, S. Park, J. Edwards, K.C. Yeung, Clinical implications for loss or diminution of expression of Raf-1 kinase inhibitory protein and its phosphorylated form in ductal breast cancer, Am. J. Cancer Res. 3 (2013) 446–464.
[12] O. Martinho, F. Pinto, S. Granja, V. Miranda-Gonçalves, M.A. Moreira, L. F. Ribeiro, C. di Loreto, M.R. Rosner, A. Longatto-Filho, R.M. Reis, RKIP inhibition in cervical cancer is associated with higher tumor aggressive behavior and resistance to cisplatin therapy, PloS One 8 (2013) e59104, https://doi.org/10.1371/journal.pone.0059104.
[13] A.E. Yesilkanal, M.R. Rosner, Targeting Raf kinase inhibitory protein regulation and function, Cancers (Basel) 10 (2018) 306, https://doi.org/10.3390/cancers10090306.
[14] S. Dangi-Garimella, J. Yun, E.M. Eves, M. Newman, S.J. Erkeland, S. M. Hammond, A.J. Minn, M.R. Rosner, Raf kinase inhibitory protein suppresses a metastasis signalling cascade involving LIN28 and let-7, EMBO J. 28 (2009) 347–358, https://doi.org/10.1038/emboj.2008.294.
[15] H.Z. Li, Y. Gao, X.L. Zhao, Y.X. Liu, B.C. Sun, J. Yang, Z. Yao, Effects of raf kinase inhibitor protein expression on metastasis and progression of human breast cancer, Mol. Cancer Res. 7 (2009) 832–840, https://doi.org/10.1158/1541-7786.MCR-08-0403.
[16] S. Baritaki, A. Chapman, K. Yeung, D.A. Spandidos, M. Palladino, B. Bonavida, Inhibition of epithelial to mesenchymal transition in metastatic prostate cancer cells by the novel proteasome inhibitor, NPI-0052: pivotal roles of snail repression and RKIP induction, Oncogene 28 (2009) 3573–3585, https://doi.org/10.1038/onc.2009.214.
[17] S. Hagan, F. Al-Mulla, E. Mallon, K. Oien, R. Ferrier, B. Gusterson, J.J. García, W. Kolch, Reduction of Raf-1 kinase inhibitor protein expression correlates with breast cancer metastasis, Clin. Cancer Res. 11 (2005) 7392–7397, https://doi.org/10.1158/1078-0432.CCR-05-0283.
[18] B. Zhang, O. Wang, J. Qin, S. Liu, S. Sun, H. Liu, J. Kuang, G. Jiang, W. Zhang, cis-acting elements and trans-acting factors in the transcriptional regulation of raf kinase inhibitory protein expression, PloS One 8 (2013) e83097, https://doi.org/10.1371/journal.pone.0083097.

[19] I. Datar, H. Tegegne, K. Qin, F. Al-Mulla, M.S. Bitar, R.J. Trumbly, K.C. Yeung, Genetic and epigenetic control of RKIP transcription, Crit. Rev. Oncog. 19 (2014) 417–430, https://doi.org/10.1615/critrevoncog.2014012025.

[20] F. Al-Mulla, S. Hagan, W. Al-Ali, S.P. Jacob, A.I. Behbehani, M.S. Bitar, A. Dallol, W. Kolch, Raf kinase inhibitor protein: mechanism of loss of expression and association with genomic instability, J. Clin. Pathol. 61 (2008) 524–529, https://doi.org/10.1136/jcp.2007.046987 (PMID: 18375747).

[21] G. Ren, S. Baritaki, H. Marathe, J. Feng, S. Park, S. Beach, P.S. Bazeley, A. B. Beshir, G. Fenteany, R. Mehra, S. Daignault, F. Al-Mulla, E. Keller, B. Bonavida, I. de la Serna, K.C. Yeung, Polycomb protein EZH2 regulates tumor invasion via the transcriptional repression of the metastasis suppressor RKIP in breast and prostate cancer, Cancer Res. 72 (2012) 3091–3104, https://doi.org/10.1158/0008-5472.CAN-11-3546.

[22] H. Zhang, J. Wu, J.M. Keller, K. Yeung, E.T. Keller, Z. Fu, Transcriptional regulation of RKIP expression by androgen in prostate cells, Cell. Physiol. Biochem. 30 (2012) 1340–1350, https://doi.org/10.1159/000343323.

[23] S. Hatzl, O. Geiger, M.K. Kuepper, V. Caraffini, T. Seime, T. Furlan, E. Nussbaumer, R. Wieser, M. Pichler, M. Scheideler, K. Nowek, M. Jongen-Lavrencic, F. Quehenberger, A. Wölfler, J. Troppmair, H. Sill, A. Zebisch, Increased expression of miR-23a mediates a loss of expression in the RAF kinase inhibitor protein RKIP, Cancer Res. 76 (2016) 3644–3654, https://doi.org/10.1158/0008-5472.CAN-15-3049.

[24] W. Huang, J. Liu, S. Hu, G. Shi, Z. Yong, J. Li, J. Qiu, Y. Cao, L. Yuan, miR-181a upregulation promotes radioresistance of nasopharyngeal carcinoma by targeting RKIP, Onco. Targets. Ther. 12 (2019) 10873–10884, https://doi.org/10.2147/OTT.S228800.

[25] K.C. Corbit, N. Trakul, E.M. Eves, B. Diaz, M. Marshall, M.R. Rosner, Activation of Raf-1 signaling by protein kinase C through a mechanism involving Raf kinase inhibitory protein, J. Biol. Chem. 278 (2003) 13061–13068, https://doi.org/10.1074/jbc.M210015200.

[26] S. Cross-Knorr, S. Lu, K. Perez, S. Guevara, K. Brilliant, C. Pisano, P. J. Quesenberry, M.B. Resnick, D. Chatterjee, RKIP phosphorylation and STAT3 activation is inhibited by oxaliplatin and camptothecin and are associated with poor prognosis in stage II colon cancer patients, BMC Cancer 13 (2013) 463, https://doi.org/10.1186/1471-2407-13-463.

[27] R. Shang, S. Lee, G. Senavirathne, E.C. Lai, microRNAs in action: biogenesis, function and regulation, Nat. Rev. Genet. 24 (2023) 816–833, https://doi.org/10.1038/s41576-023-00611-y.

[28] F. Longo, G. Gattuso, G. Spoto, D. Ricci, A.C.V. Vitale, A. Lavoro, S. Candido, M. Libra, L. Falzone, The multifaceted role of microRNAs in colorectal cancer: pathogenesis and therapeutic implications, Noncoding RNA Res. 14 (2025) 65–95, https://doi.org/10.1016/j.ncrna.2025.05.012.

[29] A.A. Svoronos, D.M. Engelman, F.J. Slack, OncomiR or tumor suppressor? The duplicity of microRNAs in cancer, Cancer Res. 76 (2016) 3666–3670, https://doi.org/10.1158/0008-5472.CAN-16-0359.

[30] A.A. Farooqi, Y. Li, F.H. Sarkar, The biological complexity of RKIP signaling in human cancers, Exp. Mol. Med. 47 (2015) e185, https://doi.org/10.1038/emm.2015.70.

[31] J.J. Skinner, M.R. Rosner, RKIP structure drives its function: a three-state model for regulation of RKIP, Crit. Rev. Oncog. 19 (2014) 483–488, https://doi.org/10.1615/critrevoncog.2014012001.

[32] A.E. Granovsky, M.C. Clark, D. McElheny, G. Heil, J. Hong, X. Liu, Y. Kim, G. Joachimiak, A. Joachimiak, S. Koide, M.R. Rosner, Raf kinase inhibitory protein function is regulated via a flexible pocket and novel phosphorylation-dependent mechanism, Mol. Cell. Biol. 29 (2009) 1306–1320, https://doi.org/10.1128/MCB.01271-08.

[33] K. Rajkumar, A. Nichita, P.K. Anoor, S. Raju, S.S. Singh, S. Burgula, Understanding perspectives of signalling mechanisms regulating PEBP1 function, Cell Biochem. Funct. 34 (2016) 394–403, https://doi.org/10.1002/cbf.3198.

[34] A.E. Granovsky, M.R. Rosner, Raf kinase inhibitory protein: a signal transduction modulator and metastasis suppressor, Cell Res. 18 (2008) 452–457, https://doi.org/10.1038/cr.2008.43.

[35] E.T. Keller, Z. Fu, M. Brennan, The role of Raf kinase inhibitor protein (RKIP) in health and disease, Biochem. Pharmacol. 68 (2004) 1049–1053, https://doi.org/10.1016/j.bcp.2004.04.024.

[36] B. Bonavida, RKIP: a pivotal gene product in the pathogenesis of cancer, Cancers (Basel) 14 (2022) 6092, https://doi.org/10.3390/cancers14246092.

[37] Y. Keshet, R. Seger, The MAP kinase signaling cascades: a system of hundreds of components regulates a diverse array of physiological functions, Methods Mol. Biol. 661 (2010) 3–38, https://doi.org/10.1007/978-1-60761-795-2_1.

[38] J.E.Jr. Ferrell, Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs, Trends Biochem. Sci. 21 (1996) 460–466, https://doi.org/10.1016/s0968-0004(96)20026-x.

[39] A. Plotnikov, E. Zehorai, S. Procaccia, R. Seger, The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation, Biochim. Biophys. Acta 2011 (1813) 1619–1633, https://doi.org/10.1016/j.bbamcr.2010.12.012.

[40] W. Zhang, H.T. Liu, MAPK signal pathways in the regulation of cell proliferation in mammalian cells, Cell Res. 12 (2002) 9–18, https://doi.org/10.1038/sj.cr.7290105.

[41] E.A. Elion, Routing MAP kinase cascades, Science 281 (1998) 1625–1626, https://doi.org/10.1126/science.281.5383.1625.

[42] S.A. Moodie, B.M. Willumsen, M.J. Weber, A. Wolfman, Complexes of Ras.GTP with Raf-1 and mitogen-activated protein kinase kinase, Science 260 (1993) 1658–1661, https://doi.org/10.1126/science.8503013.

[43] J.M. Kyriakis, H. App, X.F. Zhang, P. Banerjee, D.L. Brautigan, U.R. Rapp, J. Avruch, Raf-1 activates MAP kinase-kinase, Nature 358 (1992) 417–421, https://doi.org/10.1038/358417a0.

[44] J.S. Sebolt-Leopold, D.T. Dudley, R. Herrera, K. Van Becelaere, A. Wiland, R. C. Gowan, H. Tecle, S.D. Barrett, A. Bridges, S. Przybranowski, W.R. Leopold, A. R. Saltiel, Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo, Nat. Med. 5 (1999) 810–816, https://doi.org/10.1038/10533.

[45] J.Y. Fang, B.C. Richardson, The MAPK signalling pathways and colorectal cancer, Lancet Oncol. 6 (2005) 322–327, https://doi.org/10.1016/S1470-2045(05)70168-6.

[46] K. Yeung, P. Janosch, B. McFerran, D.W. Rose, H. Mischak, J.M. Sedivy, W. Kolch, Mechanism of suppression of the Raf/MEK/extracellular signal-regulated kinase pathway by the raf kinase inhibitor protein, Mol. Cell. Biol. 20 (2000) 3079–3085, https://doi.org/10.1128/MCB.20.9.3079-3085.2000.

[47] N. Trakul, R.E. Menard, G.R. Schade, Z. Qian, M.R. Rosner, Raf kinase inhibitory protein regulates Raf-1 but not B-Raf kinase activation, J. Biol. Chem. 280 (2005) 24931–24940, https://doi.org/10.1074/jbc.M413929200.

[48] S. Park, O. Rath, S. Beach, X. Xiang, S.M. Kelly, Z. Luo, W. Kolch, K.C. Yeung, Regulation of RKIP binding to the N-region of the Raf-1 kinase, FEBS Lett. 580 (2006) 6405–6412, https://doi.org/10.1016/j.febslet.2006.10.054.

[49] S. Ahmad, M. Abbas, M.F. Ullah, M.H. Aziz, O. Beylerli, M.A. Alam, M.A. Syed, S. Uddin, A. Ahmad, Long non-coding RNAs regulated NF-κB signaling in cancer metastasis: micromanaging by not so small non-coding RNAs, Semin. Cancer Biol. 85 (2022) 155–163, https://doi.org/10.1016/j.semcancer.2021.07.015.

[50] Y. Xia, S. Shen, I.M. Verma, NF-κB, an active player in human cancers, Cancer Immunol. Res. 2 (2014) 823–830, https://doi.org/10.1158/2326-6066.CIR-14-0112.

[51] S. Huang, C.A. Pettaway, H. Uehara, C.D. Bucana, I.J. Fidler, Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis, Oncogene 20 (2001) 4188–4197, https://doi.org/10.1038/sj.onc.1204535.

[52] A. Al-Roub, N. Akhter, F. Al-Rashed, A. Wilson, F. Alzaid, F. Al-Mulla, S. Sindhu, R. Ahmad, TNFα induces matrix metalloproteinase-9 expression in monocytic cells through ACSL1/JNK/ERK/NF-kB signaling pathways, Sci. Rep. 13 (2023) 14351, https://doi.org/10.1038/s41598-023-41514-6.

[53] T. Yamada, M. Tsuda, T. Wagatsuma, Y. Fujioka, M. Fujioka, A.O. Satoh, K. Horiuchi, S. Nishide, A. Nanbo, Y. Totsuka, H. Haga, S. Tanaka, M. Shindoh, Y. Ohba, Receptor activator of NF-κB ligand induces cell adhesion and integrin α2 expression via NF-κB in head and neck cancers, Sci. Rep. 6 (2016) 23545, https://doi.org/10.1038/srep23545.

[54] C.W. Li, W. Xia, L. Huo, S.O. Lim, Y. Wu, J.L. Hsu, C.H. Chao, H. Yamaguchi, N. K. Yang, Q. Ding, Y. Wang, Y.J. Lai, A.M. LaBaff, T.J. Wu, B.R. Lin, M.H. Yang, G. N. Hortobagyi, M.C. Hung, Epithelial-mesenchymal transition induced by TNF-α requires NF-κB-mediated transcriptional upregulation of Twist1, Cancer Res. 72 (2012) 1290–1300, https://doi.org/10.1158/0008-5472.CAN-11-3123.

[55] Y. Wu, J. Deng, P.G. Rychahou, S. Qiu, B.M. Evers, B.P. Zhou, Stabilization of snail by NF-kappaB is required for inflammation-induced cell migration and invasion, Cancer Cell 15 (2009) 416–428, https://doi.org/10.1016/j.ccr.2009.03.016.

[56] T. Collins, M.A. Read, A.S. Neish, M.Z. Whitley, D. Thanos, T. Maniatis, Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers, FASEB J. 9 (1995) 899–909.

[57] H. Wang, E. Hertlein, N. Bakkar, H. Sun, S. Acharyya, J. Wang, M. Carathers, R. Davuluri, D.C. Guttridge, NF-kappaB regulation of YY1 inhibits skeletal myogenesis through transcriptional silencing of myofibrillar genes, Mol. Cell. Biol. 27 (2007) 4374–4387, https://doi.org/10.1128/MCB.02020-06.

[58] M.B. Palmer, P. Majumder, J.C. Cooper, H. Yoon, P.A. Wade, J.M. Boss, Yin yang 1 regulates the expression of snail through a distal enhancer, Mol. Cancer Res. 7 (2009) 221–229, https://doi.org/10.1158/1541-7786.MCR-08-0229.

[59] B. Bonavida, S. Baritaki, The novel role of Yin Yang 1 in the regulation of epithelial to mesenchymal transition in cancer via the dysregulated NF-κB/snail/YY1/RKIP/PTEN circuitry, Crit. Rev. Oncog. 16 (2011) 211–226, https://doi.org/10.1615/critrevoncog.v16.i3-4.50.

[60] H. Tang, S. Park, S.C. Sun, R. Trumbly, G. Ren, E. Tsung, K.C. Yeung, RKIP inhibits NF-kappaB in cancer cells by regulating upstream signaling components of the IkappaB kinase complex, FEBS Lett. 584 (2010) 662–668, https://doi.org/10.1016/j.febslet.2009.12.051.

[61] S. Baritaki, E. Suzuki, K. Umezawa, D.A. Spandidos, J. Berenson, T.R. Daniels, M. L. Penichet, A.R. Jazirehi, M. Palladino, B. Bonavida, Inhibition of Yin Yang 1-dependent repressor activity of DR5 transcription and expression by the novel proteasome inhibitor NPI-0052 contributes to its TRAIL-enhanced apoptosis in cancer cells, J. Immunol. 180 (2008) 6199–6210, https://doi.org/10.4049/jimmunol.180.9.6199.

[62] S. Baritaki, A. Katsman, D. Chatterjee, K.C. Yeung, D.A. Spandidos, B. Bonavida, Regulation of tumor cell sensitivity to TRAIL-induced apoptosis by the metastatic suppressor Raf kinase inhibitor protein via Yin Yang 1 inhibition and death receptor 5 up-regulation, J. Immunol. 179 (2007) 5441–5453, https://doi.org/10.4049/jimmunol.179.8.5441.

[63] D. Chatterjee, Y. Bai, Z. Wang, S. Beach, S. Mott, R. Roy, C. Braastad, Y. Sun, A. Mukhopadhyay, B.B. Aggarwal, J. Darnowski, P. Pantazis, J. Wyche, Z. Fu, Y. Kitagwa, E.T. Keller, J.M. Sedivy, K.C. Yeung, RKIP sensitizes prostate and breast cancer cells to drug-induced apoptosis, J. Biol. Chem. 279 (2004) 17515–17523, https://doi.org/10.1074/jbc.M313816200.

[64] T.Y. Lee, C.L. Liu, Y.C. Chang, S. Nieh, Y.S. Lin, S.W. Jao, S.F. Chen, T.Y. Liu, Increased chemoresistance via snail-Raf kinase inhibitor protein signaling in

colorectal cancer in response to a nicotine derivative, Oncotarget 7 (2016) 23512–23520, https://doi.org/10.18632/oncotarget.8049.
[65] S. Baritaki, A. Zaravinos, Cross-talks between RKIP and YY1 through a multilevel bioinformatics pan-cancer analysis, Cancers (Basel) 15 (2023) 4932, https://doi.org/10.3390/cancers15204932.
[66] A. Zaravinos, B. Bonavida, E. Chatzaki, S. Baritaki, RKIP: a key regulator in tumor metastasis initiation and resistance to apoptosis: therapeutic targeting and impact, Cancers (Basel) 10 (2018) 287, https://doi.org/10.3390/cancers10090287.
[67] B. Bonavida, RKIP-mediated chemo-immunosensitization of resistant cancer cells via disruption of the NF-κB/snail/YY1/RKIP resistance-driver loop, Crit. Rev. Oncog. 19 (2014) 431–445, https://doi.org/10.1615/critrevoncog.2014011929.
[68] B. Bonavida, A. Jazirehi, M.I. Vega, S. Huerta-Yepez, S. Baritaki, Roles each of snail, Yin Yang 1 and RKIP in the regulation of tumor cells chemo-immuno-resistance to apoptosis, For. Immunopathol. Dis. Therap. 4 (2013) https://doi.org/10.1615/ForumImmunDisTher.2013008299, https://doi.org/10.1615/ForumImmunDisTher.2013008299.
[69] S. Li, T. Liu, W. Mo, Q. Hou, Y. Zhou, M. Liu, Z. He, Z. Liu, Q. Chen, H. Wang, X. Guo, W. Xia, M. Zeng, H. Zhao, Prognostic value of phosphorylated Raf kinase inhibitory protein at serine 153 and its predictive effect on the clinical response to radiotherapy in nasopharyngeal carcinoma, Radiat. Oncol. 11 (2016) 121, https://doi.org/10.1186/s13014-016-0696-5.
[70] S. Huerta-Yepez, N.K. Yoon, A. Hernandez-Cueto, V. Mah, C.M. Rivera-Pazos, D. Chatterjee, M.I. Vega, E.L. Maresh, S. Horvath, D. Chia, B. Bonavida, L. Goodglick, Expression of phosphorylated raf kinase inhibitor protein (pRKIP) is a predictor of lung cancer survival, BMC Cancer 11 (2011) 259, https://doi.org/10.1186/1471-2407-11-259.
[71] S. Baritaki, S. Huerta-Yepez, M.D. Cabrava-Haimandez, M. Sensi, S. Canevari, M. Libra, M. Penichet, H. Chen, J.R. Berenson, B. Bonavida, Unique pattern of overexpression of Raf-1 kinase inhibitory protein in its inactivated phosphorylated form in human multiple myeloma, For. Immunopathol Dis. Therap. 2 (2011) https://doi.org/10.1615/ForumImmunDisTher.v2.i2.90, https://doi.org/10.1615/ForumImmunDisTher.v2.i2.90.
[72] T.M. Thornton, G. Pedraza-Alva, B. Deng, C.D. Wood, A. Aronshtam, J. L. Clements, G. Sabio, R.J. Davis, D.E. Matthews, B. Doble, M. Rincon, Phosphorylation by p38 MAPK as an alternative pathway for GSK3beta inactivation, Science 320 (2008) 667–670, https://doi.org/10.1126/science.1156037.
[73] J. Azare, K. Leslie, H. Al-Ahmadie, W. Gerald, P.H. Weinreb, S.M. Violette, J. Bromberg, Constitutively activated Stat3 induces tumorigenesis and enhances cell motility of prostate epithelial cells through integrin beta 6, Mol. Cell. Biol. 27 (2007) 4444–4453, https://doi.org/10.1128/MCB.02404-06.
[74] A. Méndez-Clemente, A. Bravo-Cuellar, S. González-Ochoa, M. Santiago-Mercado, L. Palafox-Mariscal, L. Jave-Suárez, F. Solorzano-Ibarra, M. Villaseñor-García, P. Ortiz-Lazareno, G. Hernández-Flores, Dual STAT-3 and IL-6R inhibition with static and tocilizumab decreases migration, invasion and proliferation of prostate cancer cells by targeting the IL-6/IL-6R/STAT-3 axis, Oncol. Rep. 48 (2022) 138, https://doi.org/10.3892/or.2022.8349.
[75] A. Wang, G. Duan, C. Zhao, Y. Gao, X. Liu, Z. Wang, W. Li, K. Wang, W. Wang, Reduced RKIP expression levels are associated with frequent non-small cell lung cancer metastasis and STAT3 phosphorylation and activation, Oncol. Lett. 13 (2017) 3039–3045, https://doi.org/10.3892/ol.2017.5846.
[76] J. Maresch, P. Birner, M. Zakharinov, K. Toumangelova-Uzeir, S. Natchev, M. Guentchev, Additive effect on survival of Raf kinase inhibitor protein and signal transducer and activator of transcription 3 in high-grade glioma, Cancer 117 (2011) 2499–2504, https://doi.org/10.1002/cncr.25799.
[77] M. Papale, G.S. Netti, G. Stallone, E. Ranieri, Understanding mechanisms of RKIP regulation to improve the development of new diagnostic tools, Cancers (Basel) 14 (2022) 5070, https://doi.org/10.3390/cancers14205070.
[78] A. Bustamante, S. Baritaki, A. Zaravinos, B. Bonavida, Relationship of signaling pathways between RKIP expression and the inhibition of EMT-inducing transcription factors SNAIL1/2, TWIST1/2 and ZEB1/2, Cancers (Basel) 16 (2024) 3180, https://doi.org/10.3390/cancers16183180.
[79] C. Lachat, M. Boyer-Guittaut, P. Peixoto, E. Hervouet, Epigenetic regulation of EMT (epithelial to mesenchymal transition) and tumor aggressiveness: a view on paradoxical roles of KDM6B and EZH2, Epigenomes 3 (2018) 1, https://doi.org/10.3390/epigenomes3010001.
[80] Z.T. Tong, M.Y. Cai, X.G. Wang, L.L. Kong, S.J. Mai, Y.H. Liu, H.B. Zhang, Y. J. Liao, F. Zheng, W. Zhu, T.H. Liu, X.W. Bian, X.Y. Guan, M.C. Lin, M.S. Zeng, Y. X. Zeng, H.F. Kung, D. Xie, EZH2 supports nasopharyngeal carcinoma cell aggressiveness by forming a co-repressor complex with HDAC1/HDAC2 and snail to inhibit E-cadherin, Oncogene 31 (2012) 583–594, https://doi.org/10.1038/onc.2011.254.
[81] J. Lee, C. Olivieri, C. Ong, L.R. Masterson, S. Gomes, B.S. Lee, F. Schaefer, K. Lorenz, G. Veglia, M.R. Rosner, Raf kinase inhibitory protein regulates the cAMP-dependent protein kinase signaling pathway through a positive feedback loop, Proc. Natl. Acad. Sci. U. S. A. 119 (2022) e2121867119, https://doi.org/10.1073/pnas.2121867119.
[82] T.H. Lai, M. Ahmed, J.S. Hwang, S. Zada, T.M. Pham, O. Elashkar, D.R. Kim, Transcriptional repression of Raf kinase inhibitory protein gene by metadherin during cancer progression, Int. J. Mol. Sci. 22 (2021) 3052, https://doi.org/10.3390/ijms22063052.
[83] M. Ahmed, T.H. Lai, S. Zada, J.S. Hwang, T.M. Pham, M. Yun, D.R. Kim, Functional linkage of RKIP to the epithelial to mesenchymal transition and autophagy during the development of prostate cancer, Cancers (Basel) 10 (2018) 273, https://doi.org/10.3390/cancers10080273.
[84] R. Kanwal, S. Gupta, Epigenetic modifications in cancer, Clin. Genet. 81 (2012) 303–311, https://doi.org/10.1111/j.1399-0004.2011.01809.x.
[85] H. Wei, Z. Liu, H. She, B. Liu, J. Gu, D. Wei, X. Zhang, J. Wang, S. Qi, F. Ping, Promoter methylation and expression of Raf kinase inhibitory protein in esophageal squamous cell carcinoma, Oncol. Lett. 13 (2017) 1866–1872, https://doi.org/10.3892/ol.2017.5617.
[86] A. Lavoro, D. Ricci, G. Gattuso, F. Longo, G. Spoto, A.C.V. Vitale, M.C. Giuliana, L. Falzone, M. Libra, S. Candido, Recent advances on gene-related DNA methylation in cancer diagnosis, prognosis, and treatment: a clinical perspective, Clin. Epigenetics 17 (2025) 76, https://doi.org/10.1186/s13148-025-01884-2.
[87] W. Guo, Z. Dong, Y. Guo, X. Lin, Z. Chen, G. Kuang, Z. Yang, Aberrant methylation and loss expression of RKIP is associated with tumor progression and poor prognosis in gastric cardia adenocarcinoma, Clin. Exp. Metastasis 30 (2013) 265–275, https://doi.org/10.1007/s10585-012-9533-x.
[88] C. Figy, A. Guo, V.R. Fernando, S. Furuta, F. Al-Mulla, K.C. Yeung, Changes in expression of tumor suppressor gene RKIP impact how cancers interact with their complex environment, Cancers (Basel) 15 (2023) 958, https://doi.org/10.3390/cancers15030958.
[89] D.X. Li, H.Y. Cai, X. Wang, Y.L. Feng, S.W. Cai, Promoter methylation of Raf kinase inhibitory protein: a significant prognostic indicator for patients with gastric adenocarcinoma, Exp. Ther. Med. 8 (2014) 844–850, https://doi.org/10.3892/etm.2014.1833.
[90] G.E. Kim, N.I. Kim, J.S. Lee, M.H. Park, J.H. Yoon, Reduced RKIP expression is associated with breast neoplastic progression and is correlated with poor outcomes and aberrant methylation in breast carcinoma, Appl. Immunohistochem. Mol. Morphol. 25 (2017) 467–474, https://doi.org/10.1097/PAI.0000000000000323.
[91] H. Cessna, S. Baritaki, A. Zaravinos, B. Bonavida, The role of RKIP in the regulation of EMT in the tumor microenvironment, Cancers (Basel) 14 (2022) 4596, https://doi.org/10.3390/cancers14194596.
[92] I. Zlobec, M. Bihl, A. Foerster, A. Rufle, A. Lugli, Comprehensive analysis of CpG island methylator phenotype (CIMP)-high, -low, and -negative colorectal cancers based on protein marker expression and molecular features, J. Pathol. 225 (2011) 336–343, https://doi.org/10.1002/path.2879.
[93] V.H. Koelzer, E. Karamitopoulou, H. Dawson, A. Kondi-Pafiti, I. Zlobec, A. Lugli, Geographic analysis of RKIP expression and its clinical relevance in colorectal cancer, Br. J. Cancer 108 (2013) 2088–2096, https://doi.org/10.1038/bjc.2013.197.
[94] M. Labbozzetta, P. Poma, N. Vivona, A. Gulino, N. D'Alessandro, M. Notarbartolo, Epigenetic changes and nuclear factor-κB activation, but not microRNA-224, downregulate Raf-1 kinase inhibitor protein in triple-negative breast cancer SUM 159 cells, Oncol. Lett. 10 (2015) 3807–3815, https://doi.org/10.3892/ol.2015.3787.
[95] P. Poma, M. Labbozzetta, N. Vivona, R. Porcasi, N. D'Alessandro, M. Notarbartolo, Analysis of possible mechanisms accounting for raf-1 kinase inhibitor protein downregulation in hepatocellular carcinoma, OMICS 16 (2012) 579–588, https://doi.org/10.1089/omi.2012.0048.
[96] A. Zebisch, V. Caraffini, H. Sill, RAF kinase inhibitor protein in myeloid leukemogenesis, Int. J. Mol. Sci. 20 (2019) 5756, https://doi.org/10.3390/ijms20225756.
[97] Y. Zhang, Z. Sun, J. Jia, T. Du, N. Zhang, Y. Tang, Y. Fang, D. Fang, Overview of histone modification, Adv. Exp. Med. Biol. 1283 (2021) 1–16, https://doi.org/10.1007/978-981-15-8104-5_1.
[98] T. Takashina, I. Kinoshita, J. Kikuchi, Y. Shimizu, J. Sakakibara-Konishi, S. Oizumi, M. Nishimura, H. Dosaka-Akita, Combined inhibition of EZH2 and histone deacetylases as a potential epigenetic therapy for non-small-cell lung cancer cells, Cancer Sci. 107 (2016) 955–962, https://doi.org/10.1111/cas.12957.
[99] L. Inguglia, M. Notarbartolo, P. Poma, M. Labbozzetta, N. D'Alessandro, Induction of apoptosis and chemosensitization by the histone deacetylase inhibitor trichostatin in hepatocellular carcinoma cells: molecular analysis and RKIP levels, Onco. Therap. 2 (2011) 127–135, https://doi.org/10.1615/ForumImmunDisTher.v2.i2.40.
[100] R. Touboul, S. Baritaki, A. Zaravinos, B. Bonavida, RKIP pleiotropic activities in cancer and inflammatory diseases: role in immunity, Cancers (Basel) 13 (2021) 6247, https://doi.org/10.3390/cancers13246247.
[101] F. Schoentgen, S. Jonic, PEBP1/RKIP behavior: a mirror of actin-membrane organization, Cell. Mol. Life Sci. 77 (2020) 859–874, https://doi.org/10.1007/s00018-020-03455-5.
[102] J.P. Mandal, C.N. Shiue, Y.C. Chen, M.C. Lee, H.H. Yang, H.H. Chang, C.T. Hu, P. C. Liao, L.C. Hui, R.I. You, W.S. Wu, PKCδ mediates mitochondrial ROS generation and oxidation of HSP60 to relieve RKIP inhibition on MAPK pathway for HCC progression, Free Radic. Biol. Med. 63 (2021) 69–87, https://doi.org/10.1016/j.freeradbiomed.2020.12.003.
[103] Z. Xu, S. Chen, R. Liu, H. Chen, B. Xu, W. Xu, M. Chen, Circular RNA circPOLR2A promotes clear cell renal cell carcinoma progression by facilitating the UBE3C-induced ubiquitination of PEBP1 and, thereby, activating the ERK signaling pathway, Mol. Cancer 21 (2022) 146, https://doi.org/10.1186/s12943-022-01607-8.
[104] J. Yun, C.A. Frankenberger, W.L. Kuo, M.C. Boelens, E.M. Eves, N. Cheng, H. Liang, W.H. Li, H. Ishwaran, A.J. Minn, M.R. Rosner, Signalling pathway for RKIP and Let-7 regulates and predicts metastatic breast cancer, EMBO J. 30 (2011) 4500–4514, https://doi.org/10.1038/emboj.2011.312.
[105] L. Huang, T. Dai, X. Lin, X. Zhao, X. Chen, C. Wang, X. Li, H. Shen, X. Wang, MicroRNA-224 targets RKIP to control cell invasion and expression of metastasis

genes in human breast cancer cells, Biochem. Biophys. Res. Commun. 425 (2012) 127–133, https://doi.org/10.1016/j.bbrc.2012.07.025.
[106] M. Sun, S. Gomes, P. Chen, C.A. Frankenberger, D. Sankarasharma, C.H. Chung, K.K. Chada, M.R. Rosner, RKIP and HMGA2 regulate breast tumor survival and metastasis through lysyl oxidase and syndecan-2, Oncogene 33 (2014) 3528–3537, https://doi.org/10.1038/onc.2013.328.
[107] Q. Zou, H. Wu, F. Fu, W. Yi, L. Pei, M. Zhou, RKIP suppresses the proliferation and metastasis of breast cancer cell lines through up-regulation of miR-185 targeting HMGA2, Arch. Biochem. Biophys. 610 (2016) 25–32, https://doi.org/10.1016/j.abb.2016.09.007.
[108] Y. Du, X.D. Weng, L. Wang, X.H. Liu, H.C. Zhu, J. Guo, J.Z. Ning, C.C. Xiao, LncRNA XIST acts as a tumor suppressor in prostate cancer through sponging miR-23a to modulate RKIP expression, Oncotarget 8 (2017) 94358–94370, https://doi.org/10.18632/oncotarget.21719.
[109] Y. Du, X.H. Liu, H.C. Zhu, L. Wang, J.Z. Ning, C.C. Xiao, MiR-543 promotes proliferation and epithelial-mesenchymal transition in prostate cancer via targeting RKIP, Cell. Physiol. Biochem. 41 (2017) 1135–1146, https://doi.org/10.1159/000464120.
[110] J. Li, Y. Wang, Y. Song, Z. Fu, W. Yu, miR-27a regulates cisplatin resistance and metastasis by targeting RKIP in human lung adenocarcinoma cells, Mol. Cancer 13 (2014) 193, https://doi.org/10.1186/1476-4598-13-193.
[111] H. Li, R. Ouyang, Z. Wang, W. Zhou, H. Chen, Y. Jiang, Y. Zhang, H. Li, M. Liao, W. Wang, M. Ye, Z. Ding, X. Feng, J. Liu, B. Zhang, MiR-150 promotes cellular metastasis in non-small cell lung cancer by targeting FOXO4, Sci. Rep. 6 (2016) 39001, https://doi.org/10.1038/srep39001.
[112] Z. Gong, X. Chen, Y. Zhang, C. Liu, Z. Wang, X. Xu, J. Zhu, T. Xue, LncRNA GATA6-AS1 inhibits the progression of non-small cell lung cancer via repressing microRNA-543 to up-regulating RKIP, Cancer Manag. Res. 12 (2020) 9327–9338, https://doi.org/10.2147/CMAR.S254184.
[113] Z. Lin, W. Huang, Z. Xie, Y. Yi, Z. Li, Expression, clinical significance, immune infiltration, and regulation network of miR-3940-5p in lung adenocarcinoma based on bioinformatic analysis and experimental validation, Int. J. Gen. Med. 15 (2022) 6451–6464, https://doi.org/10.2147/IJGM.S375761.
[114] H. Liu, P. Li, B. Li, P. Sun, J. Zhang, B. Wang, B. Jia, RKIP suppresses gastric cancer cell proliferation and invasion and enhances apoptosis regulated by microRNA-224, Tumour Biol. 35 (2014) 10095–10103, https://doi.org/10.1007/s13277-014-2303-4.
[115] Y. Li, Z. Tian, Y. Tan, G. Lian, S. Chen, S. Chen, J. Li, X. Li, K. Huang, Y. Chen, Bmi-1-induced miR-27a and miR-155 promote tumor metastasis and chemoresistance by targeting RKIP in gastric cancer, Mol. Cancer 19 (2020) 109, https://doi.org/10.1186/s12943-020-01229-y.
[116] K. Hu, X. Qin, Y. Shao, Y. Zhou, G. Ye, S. Xu, Circular RNA MTO1 suppresses tumorigenesis of gastric carcinoma by sponging miR-3200-5p and targeting PEBP1, Mol. Cell. Probes 52 (2020) 101562, https://doi.org/10.1016/j.mcp.2020.101562.
[117] W. Li, Z.X. Yu, B.F. Ma, The increase of miR-27a affects the role of cisplatin on proliferation and migration capacities of liver cancer cells, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 5490–5498, https://doi.org/10.26355/eurrev_201809_15809.
[118] Z. Chen, Q. Cheng, Z. Ma, H. Xi, R. Peng, B. Jiang, Overexpression of RKIP inhibits cell invasion in glioma cell lines through upregulation of miR-98, Biomed. Res. Int. 2013 (2013) 695179, https://doi.org/10.1155/2013/695179.
[119] H. Kang, D. Ma, J. Zhang, J. Zhao, M. Yang, MicroRNA-18a induces epithelial-mesenchymal transition like cancer stem cell phenotype via regulating RKIP pathway in pancreatic cancer, Ann. Transl. Med. 8 (2020) 433, https://doi.org/10.21037/atm.2020.03.195.
[120] L. Yang, H. Yin, Y. Chen, C. Pan, H. Hang, Y. Lu, W. Ma, X. Li, W. Gan, H. Guo, D. Li, Low expression of PEBP1P2 promotes metastasis of clear cell renal cell carcinoma by post-transcriptional regulation of PEBP1 and KLF13 mRNA, Exp. Hematol. Oncol. 11 (2022) 87, https://doi.org/10.1186/s40164-022-00346-2.
[121] T.H. Lai, M. Ahmed, J.S. Hwang, M.E. Bahar, T.M. Pham, J. Yang, W. Kim, R. F. Maulidi, D.K. Lee, D.H. Kim, H.J. Kim, D.R. Kim, Manipulating RKIP reverses the metastatic potential of breast cancer cells, Front. Oncol. 13 (2023) 1189350, https://doi.org/10.3389/fonc.2023.1189350.
[122] V.N. Bach, J. Ding, M. Yeung, T. Conrad, H.N. Odeh, P. Cubberly, C. Figy, H. F. Ding, R. Trumbly, K.C. Yeung, A negative regulatory role for RKIP in breast cancer immune response, Cancers (Basel) 14 (2022) 3605, https://doi.org/10.3390/cancers14153605.
[123] E.T. Keller, Role of Raf kinase inhibitor protein in pathophysiology of prostate cancer, For. Immunopathol. Dis. Therap. 2 (2011) 89–94, https://doi.org/10.1615/ForumImmunDisTher.v2.i1.
[124] A. Raquel-Cunha, D. Cardoso-Carneiro, R.M. Reis, O. Martinho, Current status of Raf kinase inhibitor protein (RKIP) in lung cancer: behind RTK signaling, Cells 8 (2019) 442, https://doi.org/10.3390/cells8050442.
[125] R. Gasparri, M. Papale, A. Sabalic, V. Catalano, A. Deleonardis, F. De Luca, E. Ranieri, L. Spaggiari, Circulating RKIP and pRKIP in early-stage lung cancer: results from a pilot study, J. Clin. Med. 13 (2024) 5830, https://doi.org/10.3390/jcm13195830.
[126] S. Vivarelli, L. Falzone, C.M. Grillo, B. Bonavida, C. Crimi, I. La Mantia, M. Libra, Computational analyses of YY1 and its target RKIP reveal their diagnostic and prognostic roles in lung cancer, Cancers (Basel) 14 (2022) 922, https://doi.org/10.3390/cancers14040922.
[127] Y. Fujimori, M. Inokuchi, Y. Takagi, K. Kato, K. Kojima, K. Sugihara, Prognostic value of RKIP and p-ERK in gastric cancer, J. Exp. Clin. Cancer Res. 31 (2012) 30, https://doi.org/10.1186/1756-9966-31-30.
[128] B. Jia, H. Liu, Q. Kong, B. Li, RKIP expression associated with gastric cancer cell invasion and metastasis, Tumour Biol. 33 (2012) 919–925, https://doi.org/10.1007/s13277-012-0317-3.
[129] X.M. Zhang, H. Gu, L. Yan, G.Y. Zhang, RKIP inhibits the malignant phenotypes of gastric cancer cells, Neoplasma 60 (2013) 196–202, https://doi.org/10.4149/neo_2013_026.
[130] X. Wu, Y. Yang, Z. Xu, J. Li, B. Yang, N. Feng, Y. Zhang, S. Wang, Raf kinase inhibitor protein mediated signaling inhibits invasion and metastasis of hepatocellular carcinoma, Biochim. Biophys. Acta 2016 (1860) 384–391, https://doi.org/10.1016/j.bbagen.2015.06.009.
[131] E.J. Walker, S.A. Rosenberg, J.R. Wands, M. Kim, Role of Raf kinase inhibitor protein in hepatocellular carcinoma, For. Immunopathol. Dis. Therap. 2 (2011) 195–204, https://doi.org/10.1615/ForumImmunDisTher.v2.i2.110.
[132] Q. Huang, C. Liang, L. Wei, J. Nie, S. Lu, C. Lu, L. Zhuo, Z. Lu, X. Lin, Raf kinase inhibitory protein down-expression exacerbates hepatic fibrosis in vivo and in vitro, Cell. Physiol. Biochem. 40 (2016) 49–61, https://doi.org/10.1159/000452524.
[133] A.A. Seyhan, Trials and tribulations of MicroRNA therapeutics, Int. J. Mol. Sci. 25 (2024) 1469, https://doi.org/10.3390/ijms25031469.
[134] A.G. Seto, X. Beatty, J.M. Lynch, M. Hermreck, M. Tetzlaff, M. Duvic, A. L. Jackson, Cobomarsen, an oligonucleotide inhibitor of miR-155, co-ordinately regulates multiple survival pathways to reduce cellular proliferation and survival in cutaneous T-cell lymphoma, Br. J. Haematol. 2183 (2018) 428–444, https://doi.org/10.1111/bjh.15547.
[135] A.M. James, J. Ruckman, L.A. Pestano, R.D. Hopkins, R.C. Rodgers, W. A. Marshall, P. Rubin, D. Escolar, SOLAR: a phase 2, global, randomized, active comparator study to investigate the efficacy and safety of cobomarsen in subjects with mycosis fungoides (MF), Hematol. Oncol. 37 (2019) 562–563, https://doi.org/10.1002/hon.10_2632.
[136] C. Querfeld, T. Pacheco, F.M. Foss, A.S. Halwani, P. Porcu, A.G. Seto, J. Ruckman, M.L. Landry, A.L. Jackson, L.A. PestanO, B.A. Dickinson, M. Sanseverino, D. M. Rodman, G. Gordon, W. Marshall, Preliminary results of a phase 1 trial evaluating MRG-106, a synthetic microRNA antagonist (LNA antimiR) of microRNA-155, in patients with CTCL, Blood 128 (2016) 1829, https://doi.org/10.1182/blood.V128.22.1829.1829.