Chapter 5

MicroRNAs in Invasion and Metastasis in Lung Cancer

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1. Introduction

Despite advances in diagnosis and treatment, the morbidity and mortality of lung cancer remains to mount up. The key factor of cancer associated morbidity and mortality is principally attributable to the development of metastases. Cancer cells depart their normal microenvironment from the primary tumor site through complicated and multistep processes disseminate and colonize distant organs [1]. However, the cellular and molecular machinery underlying metastasis is relatively poorly understood so far. In order to resist cancer dissemination, more effective therapeutic strategies are clearly required.

Cellular migration and invasion mechanism are commonly thought to be associated with Rho family GTPases [2-4], JAK-STAT [5-7], MAPK [8-10], Wnt [11-13], Notch pathway [14-16]. Recently, epithelial–mesenchymal transition (EMT) programs have become the focus of the mechanism of metastasis [1, 17-20]. EMT is an embryologically conserved genetic program by which epithelial cells down regulate intercellular tight junctions, loose polarity, express mesenchymal markers, and manifest a migratory phenotype [1]. In the EMT process, Rho family GTPases [21], JAK-STAT [22], MAPK [23], Wnt [24] and Notch [25] pathways may also play an important role. In recent years, emerging studies have highlighted the critical role of these pathways and their regulation by microRNAs (miRNAs) in cancer invasion and metastasis.

MiRNAs, short (18-24 nucleotides) non-coding RNAs, are derived from long transcripts pri-miRNAs and pre-miRNAs [26-30]. By targeting 3′ untranslated regions (3′UTRs) of cognate mRNAs, miRNAs post-transcriptionally regulate gene expression and induce translational repression [29, 30]. Their specificity is determined by nucleotides 2–8 at the 5′ end, termed the miRNA "seed sequence". To date, 1527 human miRNAs have been identified (Sanger miRBase 18 http://www.miRbase.org/index. shtml), forming less than 1% of all human genes, potentially regulating more than 10% of all protein coding genes [1]. Recently, miRNAs have

been discovered to play important roles in the invasion and metastasis of malignant tumors. [31-33]. Understanding specific characteristics of miRNAs would probably serve as predictive markers and as therapeutic strategies for patients with metastasis.

In light of these recent discoveries, the present article discusses how invasion and EMT pathways are regulated by miRNAs. We have classified invasion programs and key proteins involved in EMT according to the signaling pathway showed above and point out validated miRNAs regulating their expression and highlight critical knowledge gaps that remain to be addressed to enable improved understanding of the molecular mechanisms behind EMT and metastasis. A list of experimentally validated miRNAs regulating key proteins involved in invasion–metastasis programs or participating in some principal pathways can be found in Figure 1.

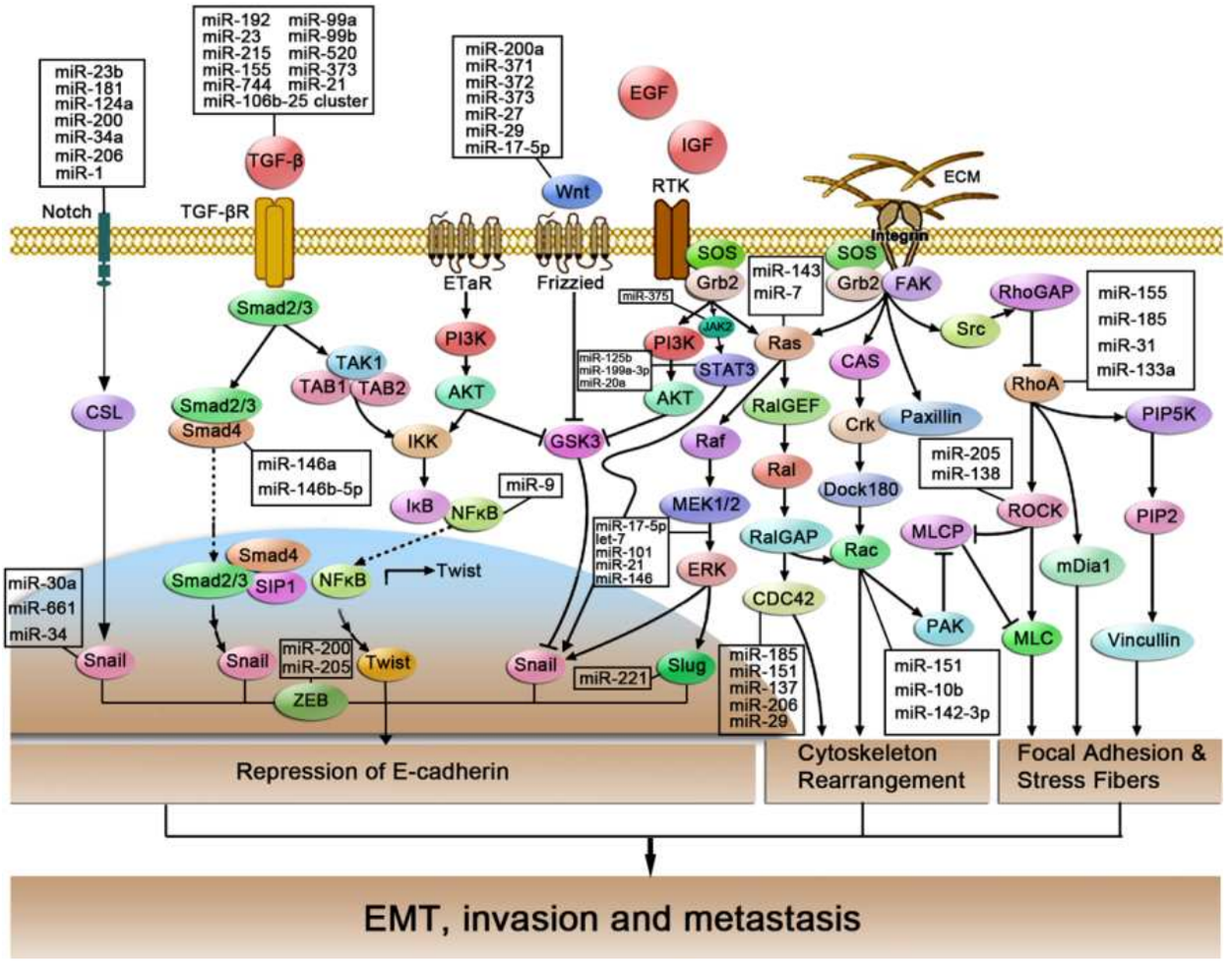

Figure 1. The experimentally validated miRNAs regulate key proteins involved in invasion–metastasis programs or participating in some principal pathways.

2. Rho family of GTPases

The Rho family of GTPases, a family of small (~21 kDa) signaling G protein, is a subfamily of the Ras superfamily [34]. In mammals, the Rho family is made of 20 members distributed

into eight subfamilies: Rho, Rac, Cdc42, Rnd, RhoU/V, RhoBTB, RhoH and RhoD/F. Almost all research involves the three most common members of the Rho family: Cdc42, Rac1 and RhoA [35]. Over expression of Rho GTPases is associated with reorganization of actin cytoskeleton, which plays an important role in cell migration, invasion and metastasis that are important aspects of cancer progression [36].

Emerging studies have indicated that miRNAs participate in the Rho GTPases signaling pathway. Among the tested miRNAs, the present articles demonstrated that miR-155, miR-185, miR-31 and miR-133a are associated with RhoA in cell migration and invasion. MiR-155 may play an important role in TGF-β-induced EMT and cell migration and invasion by targeting RhoA [37]. MiR-185 is a negative regulator of RhoA and Cdc42, and could inhibit proliferation and invasion of colorectal cancer cells [38]. The Effects of miR-31 on metastasis may be associated with concurrent suppression of integrin alpha 5, radixin, and RhoA phenocopies [39]. Chiba and his colleagues reported that RhoA expression is negatively regulated by miR-133a in bronchial smooth muscle cells [40].

Moreover, some studies discussed the regulation of cell migration and invasion by miRNA may be attribute to Rho-associated serine-threonine protein kinase (ROCK), one of the best characterized downstream effectors of Rho, that is activated when it selectively binds to the active GTP-bound form of Rho [41, 42]. As with Rho, ROCK has been implicated in altering cell migration and invasion during tumor cell metastasis [43, 44]. Yu and his colleagues indicate that downregulation of miR-205 resulted in an increase in Rho-ROCKI activity, phosphorylation of the actin severing protein cofilin, and a corresponding diminution of filamentous actin [45].

A number of articles reported that some miRNAs regulate cell migration and invasion by targeting Rac and Cdc42. Recently, microRNA-142-3p, a new regulator of Rac1, suppresses the migration and invasion of hepatocellular carcinoma cells [46]. The regulation of cancer cell migration by MiR-10b may be attribute to activate Rac by targets Tiam1 [47]. MiR-151 exerts this function by directly targeting RhoGDIA, a putative metastasis suppressor in hepatocellular carcinoma (HCC), thus leading to the activation of Rac1, Cdc42 and Rho GTPases [48]. Liu and his colleagues have found that miR-137 may have a tumor suppressor function by directly targeting Cdc42 to inhibit the proliferation and invasion activities of colorectal cancer cells [49, 50]. MiR-206 may suppress invasion and migration of MDA-MB-231 cells in vitro partly via regulating actin cytoskeleton remodelling by downregulating Cdc42 [51]. MiR-29 activates p53 by targeting p85 alpha and Cdc42 [52].

In addition, MiR-21 targets the tumor suppressor Rho B and regulates proliferation, invasion positively in colorectal cancer cells [53, 54]. Jiang and his colleagues have indicated that miR-138 plays an important role in tongue squamous cell carcinoma cell migration and invasion by concurrently targeting Rho C and ROCK2 [36]. Studies on the association of Rho with miRNAs highlight the importance of miRNAs in invasion and metastasis of malignant tumors.

3. JAK-STAT

The JAK-STAT signaling pathway transmits information from chemical signals outside the cell, through the cell membrane, and into gene promoters on the DNA in the cell nucleus, which causes DNA transcription and activity in the cell. JAK, short for Janus Kinase, is a family of intracellular, nonreceptor tyrosine kinases that transduce cytokine-mediated signals via the JAK-STAT pathway. As a key component of the JAK/STAT pathway, Signal Transducer and Activator of Transcription, an important transcription factors, is activated by JAK [55, 56]. In JAK and STAT family, emerging studies have indicated that JAK2/STAT3 pathway is well-established regulators of cell migration, and has been implicated in the process of tumor cell invasion and metastasis [57].

Some studies have indicated that miRNAs participate in the JAK-STAT signaling pathway. MiR-375 may function as a tumor suppressor to regulate gastric cancer cell proliferation potentially by targeting the JAK2 oncogene [58]. MiR-125b suppresses the proliferation and migration of osteosarcoma cells through downregulation of STAT3 [59]. Transfection of precursor miR-199a-3p into osteosarcoma cell lines significantly decreased cell growth and migration. Duan and his colleagues observed decreased mTOR and STAT3 expression in miR-199a-3p transfected cells [60]. Yan and his colleagues indicated that miR-20a regulates STAT3 at the post-transcriptional level, resulting in inhibition of cell proliferation and invasion of pancreatic carcinoma [61].

4. MAPK pathway

The Mitogen Activated Protein Kinase (MAPK) pathway is a frequent event in tumorigenesis. MAPKs have been implicated in cell migration, proteinase induction, apoptosis, and angiogenesis, events that are essential for successful completion of metastasis [8]. The presence of at least six MAPK in yeast suggests that there are more in mammals: extracellular signal-regulated kinases (ERK1, ERK2), c-Jun N-terminal kinases (JNKs), p38 isoforms, ERK5, ERK3/4, ERK7/8. In vivo and in vitro studies have confirmed that three major subgroups of MAPK including ERK1/2, JNK, and p38, are specifically involved in invasion and metastasis [9, 10, 62].

Mounting studies have indicated that miRNAs participate in the MAPK signaling pathway. MiR-143 plays an important role in prostate cancer proliferation, migration and chemosensitivity by suppressing KRAS and subsequent inactivation of MAPK pathway [63]. MiR-17-5p significantly activates the p38 kinase pathway [64]. Raf kinase inhibitory protein suppresses a cascade of metastasis signalling involving LIN28 and let-7 [65]. Zhu and his colleagues found that miR-101 targets MAPK phosphatase 1 to regulate the activation of MAPKs in macrophages [66]. MiR-146a suppresses tumor growth and progression by targeting EGFR pathway and in a p-ERK-dependent manner in castration-resistant prostate cancer [67]. Liu and his colleagues indicated that miR-21 induces tumor angiogenesis through targeting PTEN, leading to activate AKT and ERK1/2 signaling pathways [68,69]. EGFR promotes lung tumorigenesis by activating miR-7 through a Ras/ERK/Myc pathway that targets the ETS2 transcriptional repressor ERF [70].

5. Wnt signaling pathway

Wnt signaling pathway controls tissue polarity and cell movement through the activation of RhoA, JNK, and nemo-like kinase (NLK) signaling cascades. The Wnt gene family is a group of developmental genes that encode cysteine-rich glycosylated proteins [71]. Aberrant activation of Wnt signaling pathway in human cancer leads to more malignant phenotypes, such as abnormal tissue polarity, invasion, and metastasis [72].

A number of studies have indicated that miRNAs participate in the Wnt signaling pathway. MiR-200a is a new tumor suppressor that can regulate the activity of the Wnt/β-catenin signaling pathway [73]. MiR-371-373 expression is induced by lithium chloride and is positively correlated with Wnt/β-catenin-signaling activity in several human cancer cell lines [74]. MiR-27 directly targeted and inhibited adenomatous polyposis coli (APC) gene expression, and activated Wnt signaling through accumulation of β-catenin [75]. Kapinas and his colleagues reported that miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop [76]. MiR-17-5p plays an important role in breast cancer cell invasion and migration by suppressing HBP1 and subsequent activation of Wnt/β-catenin [77]. Kennell and his colleagues demonstrated that miR-8 family members play an evolutionarily conserved role in regulating the Wnt signaling pathway [78].

6. Notch signaling pathway

The Notch signaling pathway is a conserved ligand–receptor signaling pathway. Notch genes encode single-pass transmembrane proteins that can be activated by interacting with a family of its ligands. To date, four Notch receptors have been identified in mammals, including human, such as Notch-1-4. It has been well known that Notch signaling plays important roles in maintaining the balance involved in cell proliferation, survival, apoptosis, and differentiation which affects the development and function of many organs [79]. Therefore, dysfunction of Notch prevents differentiation, ultimately guiding undifferentiated cells toward malignant transformation. Indeed, many observations suggest that alterations in Notch signaling are associated with invasion and metastasis in many human cancers [14-16].

Mounting studies have indicated that miRNAs participate in the Notch signaling pathway. MicroRNA-23b is capable of inducing tolerogenic properties of dendritic cells in vitro through the inhibition of the Notch1 and NF-κB signalling pathways [80]. MicroRNA-181 promotes natural killer (NK) cell development by regulating Notch signaling [81]. MiR-124a mediates stroke-induced neurogenesis by targeting the JAG-Notch signaling pathway [82]. Pang and his colleagues demonstrated that miR-34a affected cell invasion by regulating expression of urokinase plasminogen activator through Notch [83]. MiR-206 targets Notch 3, activates apoptosis, and inhibits tumor cell migration and focus formation [84]. MiR-1 influences cardiac differentiation in Drosophila and regulates Notch signaling [85]. Some studies indicated that the ZEB1/miR-200 feedback loop controls Notch signalling in cancer cells [86, 87].

7. EMT

Several oncogenic pathways (Rho GTPases, JAK-STAT, MAPK, Wnt and Notch) may induce EMT [21-25]. In particular, the association of those pathways with EMT has been shown to activate EMT-inducing transcriptional regulators such as the members of the Snail family, the zinc finger transcription factors (ZEB), Transforming growth factor beta (TGF-β), Twist and Slug.

Members of the Snail family of transcriptional regulators, namely Snail 1 and Snail 2, have emerged as a key regulatory factor of EMT. The zinc finger transcription factors ZEB1 and ZEB2 also make a pivotal contribution to this regulation. TGF-β, a major inducer of EMT, exists in at least three isoforms called TGF-β1, TGF-β2 and TGF-β3. It cooperates with stem cell pathways like Wnt, Ras and Notch to induce EMT [88, 89]. Twist, a basic helix-loop-helix transcription factor, exists in at least two isoforms called Twist 1 and Twist 2. Twist proteins promote EMT by turning-down the expression of epithelial specific proteins, such as the E-cadherin and by up-regulating the expression of mesenchymal markers such as the N-cadherin, the vimentin and the smooth-muscle actin [90]. Slug, a zinc finger transcription factor, whose product belongs to the Snail family of developmental regulatory proteins, is transcriptional repressors of E-cadherin and induces EMT [1].

Emerging studies have indicated that miRNAs participate in the EMT. The miR-106b-25 cluster targets Smad7, activates TGF-β signaling, and induces EMT in human breast cancer [91]. MiR-27 promoted EMT by activating the Wnt pathway [92]. MiR-221/222 targeting of trichorhinophalangeal 1 (TRPS1) promotes EMT in breast cancer [93]. MiR-194 inhibits EMT of endometrial cancer cells by targeting oncogene BMI-1 [94]. Let-7d negatively modulates EMT expression and also plays a role in regulating chemo-resistant ability in oral cancer [95]. MiR-200b and miR-15b regulate chemotherapy-induced EMT in human tongue cancer cells by targeting BMI-1 [96]. Kumarswamy and his colleagues found that miR-30a targets Snai1, inhibits invasion and metastasis, and is downregulated in non-small cell lung cancer (NSCLC) [97]. Vetter and his colleagues indicated that miR-661 expression in Snail 1-induced EMT contributes to breast cancer cell invasion by targeting Nectin-1 and StarD10 messengers [98]. Some studies indicated that the miR-200 family and miR-205 regulate EMT by targeting ZEB1 and SIP1 [99, 100].

8. MicroRNAs in invasion and metastasis in lung cancer

Lung cancer is the leading cause of death among the malignant tumors worldwide, and the incidence of lung cancer is increasing. Tumor invasion and metastasis are the critical steps in determining the aggressive phenotype of human cancers. Mortality of tumor patients results mainly from cancer cells spreading to distant organs. In order to resist cancer dissemination, more effective therapeutic strategies are clearly required. However, the cellular and molecular machinery, underlying invasion and metastasis by miRNA in lung cancer, is relatively poorly understood. In light of these recent discoveries, we have classified the experimentally validated miRNAs regulating the invasion and metastasis of lung cancer and showed in Figure 2.

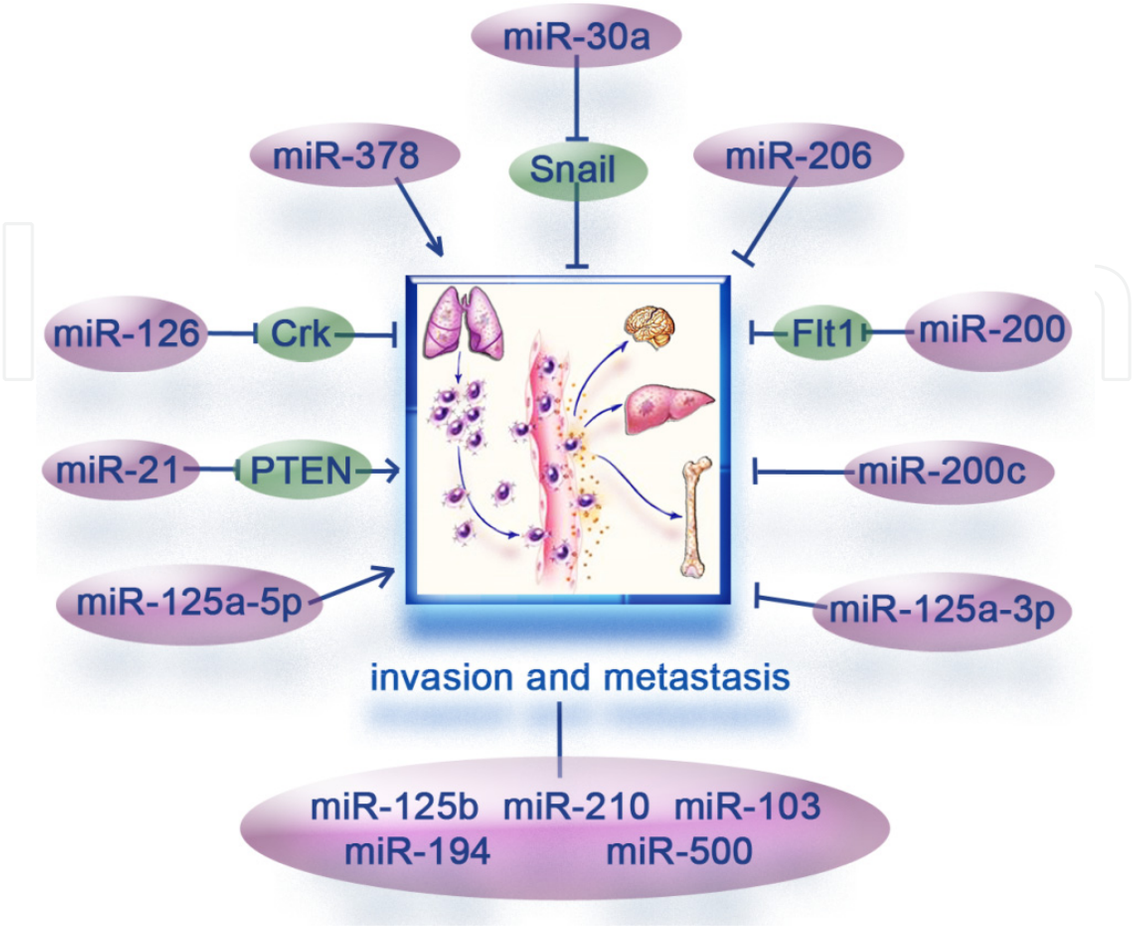

Figure 2. The experimentally validated miRNAs regulate the invasion and metastasis in lung cancer.

In light of these recent discoveries, the present article indicated that miRNAs participate in invasion and metastasis in lung cancer. Zhu and his colleagues indicated that MTA1 functions in regulating the invasive phenotype of lung cancer cells and this regulation may be through altered miRNA expression, such as miR-125b, miR-210, miR-103, miR-194 and miR-500 [101]. Hu and his colleagues reported that MiR-193b modulated proliferation, migration, and invasion of NSCLC [102]. A p53/miR-34 axis has been found that it regulates Snail1-dependent cancer cell EMT [103]. MiR-378 is associated with NSCLC brain metastasis by promoting cell migration, invasion and tumor angiogenesis [104]. MiR-30a targets Snai1, inhibits invasion and metastasis, and is downregulated in NSCLC [105]. Expression level of miR-206 was inversely correlated with metastatic potential of lung cancer [106]. Roybal and his colleagues demonstrated that miR-200 Inhibits lung adenocarcinoma cell invasion and metastasis by targeting Flt1 [107]. Loss of miR-200c expression induces an aggressive, invasive, and chemoresistant phenotype in NSCLC [108]. In our previous studies, we found that hsa-miR-125a-3p and hsa-miR-125a-5p are downregulated in NSCLC and have inverse effects on invasion and migration of lung cancer cells [109]. Zhang and his colleagues reported that miR-21 post-transcriptionally downregulates the expression of tumor suppressor PTEN and stimulates growth and invasion in NSCLC [110]. Crawford and his colleagues indicated that MiR-126 alters lung cancer cell phenotype by inhibiting adhesion,

migration, and invasion and the effects on invasion may be partially mediated through Crk regulation [111]. The deep mechanisms of miRNAs in invasion and metastasis which contribute to lung cancer are worthy of further investigation.

9. Conclusion and future perspective

Despite recent advances in diagnosis and treatment, lung cancer remains a leading cause of death among the malignant tumors worldwide, and the incidence of lung cancer is increasing. Even so, no improvement in prognosis has been observed if the patient presents with metastases at diagnosis. A better understanding of the mechanism of tumor cell invasion is critical for the development of more effective treatments for metastatic cancer. In recent years, emerging studies have attested to the association between miRNAs and the mechanism in critical processes during cancer dissemination, and we have summarized many of these in the present manuscript. Here, we have condensed much of this early work, and highlight key deregulated miRNAs targeting molecules involved in Rho family GTPases, JAK-STAT, MAPK, Wnt, Notch pathway and transcriptional control of EMT. In the future, a more complete dissection of the pathways controlled by miRNAs may offer new insights on metastasis, and highlight promising areas for the development of novel anti-cancer therapies.

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