The interplay between PP2A and microRNAs in leukemia

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Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase family whose members have been implicated in tumor suppression in many cancer models. In many cancers, loss of PP2A activity has been associated with tumorigenesis and drug resistance. Loss of PP2A results in failure to turn off survival signaling cascades that drive drug resistance such as those regulated by protein kinase B. PP2A is responsible for modulating function and controlling expression of tumor suppressors such as p53 and oncogenes such as BCL2 and MYC. Thus, PP2A has diverse functions regulating cell survival. The importance of microRNAs (miRs) is emerging in cancer biology. A role for miR regulation of PP2A is not well understood; however, recent studies suggest a number of clinically significant miRs such as miR-155 and miR-19 may include PP2A targets. We have recently found that a PP2A B subunit (B55α) can regulate a number of miRs in acute myeloid leukemia cells. The identification of a miR/PP2A axis represents a novel regulatory pathway in cellular homeostasis. The ability of miRs to suppress specific PP2A targets and for PP2A to control such miRs can add an extra level of control in signaling that could be used as a rheostat for many signaling cascades that maintain cellular homeostasis. As such, loss of PP2A or expression of miRs relevant for PP2A function could promote tumorigenesis or at least result in drug resistance. In this review, we will cover the current state of miR regulation of PP2A with a focus on leukemia. We will also briefly discuss what is known of PP2A regulation of miR expression.

Keywords: PP2A, microRNA, leukemia, signal transduction, AKT

INTRODUCTION
The cellular signaling pathways that control cellular homeostasis are complex and involve a diversity of activators and suppressors. As signal transduction is a dynamic process, the elements involved in turning off kinases are just as important as those that activate the signaling cascade. Protein phosphatase 2A (PP2A) is thought of as a global negative regulator of signaling. In reality, PP2A is not a single enzyme but rather a family of protein phosphatases that vary in the substrates they target, in the cell types where they are expressed, in the cellular compartments where they are found, and how they are regulated (1–5). The diversity of function lies in the PP2A structure. The enzyme is a heterotrimer that consists of a catalytic core that is responsible for the dephosphorylation event as well as a regulatory subunit that controls substrate specificity and cellular localization. The catalytic core is comprised of a catalytic subunit (PR55) that has two isoforms (PR55/PR61/B′; and PR72/B′; Ref. (1–4)). The importance of miRs relevant for PP2A function could promote tumorigenesis or at least result in drug resistance in the malignant cells. In cell that fails to express the PP2A isoform with B56α subunit (PPP2R5A) when phosphorylated at serine 28 re-localizes from the nucleus to the mitochondria (10). While nuclear B56α likely supports survival signaling, in the mitochondria the B subunit dephosphorylates and inactivates BCL2 to support pro-death function. Depending on phosphorylation status, the PP2A isoform with B56α can either support or impede survival (10). Thus, cell growth and survival of any given cell depends on it having the appropriate PP2A isoform(s) in place (with the required subunit modifications) to regulate the signaling cascades that are critical for its cell type. In cell that fails to express the PP2A isoforms required for its cell type, it is plausible that the result would be aberrant activation of any number of signal cascades that promotes tumorigenesis or supports drug resistance in the malignant cells. Suppression of the PP2A family to promote global activation of cellular kinases can be achieved by targeting the catalytic core. Mechanisms include post-translational modification to inactivate protein phosphatase function [e.g., phosphorylation or methylation of the catalytic subunit; Ref. (11–13)], involvement of viral inhibitors like the SV40 small T antigen (14–17), or activation of cellular inhibitors such as SET or CIP2A, the former of which is induced by BCR–ABL in chronic myeloid leukemia.
AML indicate that less than half of AML patients have a RTK mutation. Though mutation of receptor tyrosine kinases (RTK) like FLT3 has been characterized so far being found in the Aα and Aβ isoforms (24, 25). Also, chromosome deletion or translocation containing PP2A subunits have been identified. The 3q deletion can include catalytic Cα subunit and loss of this PP2A subunit has been suggested to be important in myelodysplastic syndrome (MDS) as determined by the List Laboratory (26, 27). The topics of genetic and post-translational control of PP2A and role of SET and CIP2A are covered elsewhere in this Research Topics series. This review will focus on the emerging role of microRNAs (miRs) to regulate PP2A and will also include how at least one PP2A isoform has been shown to regulate miR expression. The interplay between the protein phosphatases and miRs suggest an elaborate feedback mechanism exists to serve as an extra level of control for signal transduction.

PP2A IN ROLE IN LEUKEMIA

We have some knowledge of PP2A role in CML and Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL) thanks in large part to the work of the Perrotti group in their studies on BCR–ABL regulation of PP2A in these diseases (18–23). In CML, activation of PP2A is an important part of the mechanism of killing the malignant cells. Furthermore, activation of PP2A by FTY-720 or like drugs has anti-leukemic properties including for acute myeloid leukemia (AML) cells (18–21). Still, little is known about the role of PP2A in leukemia and other hematologic malignancies at present. AML remains a highly fatal disease despite our best efforts to develop novel therapies. Strategies to design tailored therapy have been in vogue but a problem arises that AML patients with the poorest outcome likely have multiple survival kinase cascades activated. Kornblau and colleagues reported that simultaneous activation of protein kinase B (AKT), protein kinase Cα (PKCα), and extracellular signal regulated kinase (ERK) is very detrimental to the AML patient (28). Up to 80% of AML patients have phosphorylated AKT and activation of the kinase is associated with poor prognosis (29–31). Mutations of upstream AKT signaling activators such as Fms-related tyrosine kinase (FLT3), c-KIT, or RAS are found in AML patients (29–31). Targeting mutated enzymes like FLT3 could suppress the aberrant induction of AKT. Though mutation of receptor tyrosine kinases (RTK) like FLT3 has been suggested to be essential for AML, the recent report in Cell from Welch and colleagues on genomic screening of mutations in AML indicate that less than half of AML patients have a RTK mutation suggesting that other mechanisms are necessary to activate leukemic signaling pathways (32). Gallay and colleagues determined that reduced PP2A activity was associated with increased phosphorylation of AKT in AML patient samples (33). The Odero Laboratory in Spain has done extensive studies of PP2A and SET in AML (34–36). They determined that elevated SETBP1 (which stabilizes SET and supports suppression of PP2A) was associated with poor survival outcome in AML patients (34). Like Gally et al. (33), they found that PP2A activity was reduced in AML patient samples and suppressed expression of the protein phosphatase likely involved multiple mechanisms including phosphorylation of the C subunit, inhibition by SET or CIP2A, or dysregulation of subunit expression (35). Our own work has determined that suppressed expression of a specific B subunit, B55α, is critical in AML patients resulting in shorter remission duration and increased activation of AKT and PKCα (37, 38). PP2A in acute lymphoid leukemia has not been studied as well in the clinical setting but pre-clinical models suggest that the protein phosphatase family is important in regulation of apoptosis via BCL2 and NOTCH pathways (10, 39–41).

THE ROLE OF miRS IN LEUKEMIA

The discovery of miRs was made in C. elegans in 1993 when the lin-4 gene product was identified as a RNA product that was complimentary to the lin-14 gene (42). In 2002, George Calin with Carlo Croce discovered that the 13q14 deletions found in chronic lymphoblastic leukemia (CLL) resulted in loss of miR-15 and miR-16, resulting in over expression of anti-apoptotic proteins such as BCL2 (43). An explosion of studies has occurred linking expression of a wide variety of miRs to various cancers [reviewed in Ref. (44–46)]. The role of miRs in cancer has proven to be complex. miRs can either act as tumor suppressors or tumor promoters depending on their targets (44–46). For instance, the let-7 family of miRs tends to act as tumor suppressors by targeting a number of pro-survival molecules such as RAS, BCL-XL, and MYC (46–49). Recent work from the Andreff Laboratory identified a novel mechanism for the chemokine receptor CXCR4 in the regulation of let-7 (50). In leukemia, a number of miRs have been identified that are important in leukemia cell biology and drug resistance (51–58). Some of these miRs, like miR-15, are tumor suppressors that are reduced or lost in cancer cells. miRs play a critical role in cell differentiation. There has been intensive investigation of miR-155 in a variety of leukemias (59–63). The Baltimore group found that overexpression of miR-155 in murine hematopoietic stem cells resulted in altered hematopoiesis skewing toward granulocyte/macrophage population and mice exhibited features of myeloid neoplasia (59). Importantly, the miR was found to target a number of genes that regulate myeloid differentiation including PU.1. In that and subsequent studies, miR-155 was found to be associated with poor survival outcome in AML patients (59, 61, 62), miR-181 family members, on the other hand, support myeloid differentiation by targeting homeobox (HOX) genes such as HOXA9 and other myeloid regulatory genes (64, 65). While still the role of many miRs in leukemia remains unknown, a growing number of these ncRNAs are clearly important in leukemia biology and may be considered for targeting in future therapeutic strategies.

miR REGULATION OF PP2A SUBUNITS

The B subunit regulation mechanism best studied is the one involving proteolytic control. Alternative mechanisms could include regulation of PP2A genes by transcription factors or miRs. A comprehensive study of the A α gene was done by Chen and colleagues and identified a number of transcription factors including SP-1 that control expression of the scaffold gene (66). In hepatocellular carcinoma (HCC), a single nucleotide polymorphism (SNP) mutation in the PP2A A α promoter affects regulation by NF kB (67). The study found that individuals from Southern China with the SNP (rs11453459) were less likely to develop HCC as these individuals exhibited greater expression of the PP2A scaffold gene.
mediated by NFκB (67). Other examples of gene regulation of PP2A subunits include Ikaros suppression of transcription of the Cα subunit (68). Unfortunately, little is known of how PP2A B subunit genes are transcribed.

There is an ongoing effort to characterize the regulation of PP2A subunit gene expression by miRs (69–80). miRs that have been identified as suppressors of PP2A subunit expression are listed in Table 1 and depicted in Figure 1. Also included in Table 1 and Figure 1 are miRs that have been found to target SET and CIP2A as suppression of these PP2A inhibitors would allow for full and potent PP2A activity (81, 82). Regulation of B55α by miR-222 may be important in leukemia as this miR is elevated in MDS patients and the levels of the miR were even higher in patients that progressed to AML (58). Effects on differentiation may be important as many miRs including miR-222 regulate myeloid differentiation (83–86). In addition, miR-222 has been found to be overexpressed in some groups of AML patients and CLL patients with refractory disease (56, 87). NOTCH regulation of miR-19 is important as many miRs including miR-222 regulate myeloid differentiation (83–86). In addition, miR-222 has been found to be important for promoting tumor survival and metastasis of breast cancer and cervical cancer cells (69, 70). The adenovirus type 5 E1A protein activates PP2A using C2-ceramide was shown to induce hESC differentiation, (c) hESC self-renewal is maintained in the presence of the PP2A inhibitor okadaic acid, and (d) suppression of PP2A C subunit expression by siRNA-induced stem cell genes such as OCT-4 and Nanog (95). The study found that stabilization of MYC and activation of AKT was important in maintenance of the hESC. Considering the importance of MYC (96) and AKT (30, 31) in leukemic stem cells, it is plausible that PP2A regulation of both these molecules is important in maintenance of leukemia stem cells (LSC). At present, it is unknown if miRs play a role in regulating PP2A activity in normal or cancer stem cells. An interesting possibility emerges that the Cα subunit of PP2A and miR-520h could be involved in this process. Su and colleagues recently demonstrated that miR-520h suppression of the PP2A Cα subunit is critical for promoting tumor survival and metastasis of breast cancer and cervical cancer cells (69, 70). The adenovirus type 5 E1A protein was found to act as a tumor suppressor by inhibiting miR-520h.

Table 1 | List of miRs reported that regulate PP2A subunit or regulators.

| PP2A subunit or regulator | miR identified | Cancer or disease involved | Reference |
|---------------------------|----------------|----------------------------|-----------|
| Ca (PPP2CA)               | miR-520h       | Breast cancer              | (69, 70)  |
|                           |                | Cervical cancer            |           |
| Aβ (PPP2R1B)              | miR-200c       | Esophageal cancer          | (71)      |
| B55α (PPP2R2A)            | miR-222        | Hepatocellular carcinoma   | (72)      |
| B55α (PPP2R2A)            | miR-222        | Lung cancer                | (73)      |
| B55α (PPP2R2A)            | miR-31         | Lung cancer                | (74)      |
| B56α (PPP2R5A)            | miR-155        | Infection (macrophage      | (76)      |
|                           |                | response to bacteria)      |           |
| B56α (PPP2R5E)            | miR-19α        | Acute lymphoblastic       | (77, 78)  |
|                           |                | leukemia                   |           |
| B56x (PPP2R5E)            | miR-23a        | Gastric cancer             | (79)      |
| B56y (PPP2R5C)            | miR-135        | Lymphoma                   | (80)      |
| SET                       | miR-190b       | Choriocarcinoma            | (81)      |
| CIP2A                     | miR-375        | Oral cancer                | (82)      |

Listed are reported miRs that target the PP2A catalytic core subunits A and C, various regulatory B subunits, and cellular inhibitors SET and CIP2A. Cancer or disease state associated with each report is listed.

NOVEL FEEDBACK LOOPS FOR SIGNAL TRANSDUCTION: POSSIBLE ROLE FOR miRs AND PP2A

It has been demonstrated that miR-29a is critical for myeloid differentiation and the miR has been shown to be reduced in AML (88, 89). Gong and colleagues recently identified a feedback loop involving the miR-29 family members, AKT2, and MYC (88). The authors found that miR-29 targets AKT which could contribute to the miR’s tumor suppressor activity in AML. Consentient with a role for MYC as a negative regulator of miR-29a, over expression of MYC in AML-derived cell lines resulted in suppression of miR-29a with induction of AKT2 expression while introduction of miR-29a into cells blocked MYC expression and AKT2 levels were reduced. AKT regulation of MYC is likely via suppression of AKT which regulates MYC proteolysis. However, AKT has been shown to support expression of the PP2A B subunit (i.e., B56α) that negatively regulates MYC (10, 90, 91). Thus, an elaborate feedback mechanism to regulate AKT signaling could be mediated by interplay between miR-29a, MYC, and PP2A. To further complicate matters, miR-155 has been identified as an important tumor promoter in AML, high risk MDS, and CLL (61–63, 92–94). One of the targets associated with miR-155 is SHIP 1, a member of the inositol polyphosphate-5-phosphatase family (63, 94). As SHIP 1 is a negative regulator of AKT, suppression of the phosphatase would result in activation of AKT. As miR-155 has been shown to be a prognostic factor for cytogenetic normal AML patients (62), it would be plausible that miR-155 suppression of SHIP 1 could promote signaling in patients that would likely lack activating mutations. Interestingly, miR-155 has now been shown to target B56α (76). Thus, we have another layer of complexity in the putative miR/PP2A regulatory axis to control AKT in leukemia cells. A hypothetical model is depicted in Figure 2. Though quite complex, the possible feedback regulatory mechanism could be necessary for controlling cell fate and dysregulation could ultimately contribute to leukemogenesis and/or drug resistance.

PP2A, miRs, AND CANCER STEM CELLS

A study by Yoon and colleagues using human embryonic stem cells (hESC) demonstrated that (a) PP2A activity increases with concomitant increase in A and C subunit expression in differentiating hESC, (b) introduction of catalytic C subunit into hESC or activation of PP2A using C2-ceramide was shown to induce hESC differentiation, (c) hESC self-renewal is maintained in the presence of the PP2A inhibitor okadaic acid, and (d) suppression of PP2A C subunit expression by siRNA-induced stem cell genes such as OCT-4 and Nanog (95). The study found that stabilization of MYC and activation of AKT was important in maintenance of the hESC. Considering the importance of MYC (96) and AKT (30, 31) in leukemic stem cells, it is plausible that PP2A regulation of both these molecules is important in maintenance of leukemia stem cells (LSC). At present, it is unknown if miRs play a role in regulating PP2A activity in normal or cancer stem cells. An interesting possibility emerges that the Cα subunit of PP2A and miR-520h could be involved in this process. Su and colleagues recently demonstrated that miR-520h suppression of the PP2A Cα subunit was critical for promoting tumor survival and metastasis of breast cancer and cervical cancer cells (69, 70). The adenovirus type 5 E1A protein was found to act as a tumor suppressor by inhibiting miR-520h.
expression which resulted in activation of PP2A to suppress pro-survival functions of NFκB and TWIST. Interestingly, a recent analysis of miR expression in hESC cell lines indicated that members of the miR-520 family were highly expressed in those cells (97). While it remains to be determined, it is plausible that suppression of PP2A in stem cells observed by Yoon and colleagues could be due to suppression of PP2A by miR-520h (95). As this miR targets a critical component of the catalytic core (i.e., Cα), this miR could potentially suppress any number of PP2A isoforms. Another interesting possibility involves miR-200c and the PP2A Aβ subunit. Recent studies have suggested that miR-200 family members are critical for maintaining stem cells [reviewed in Ref. (97–99)]. These miRs are positively regulated by MYC, OCT-4, and other stem cell transcription factors (98). The miR-200 family member was found to suppress the PP2A Aβ subunit in esophageal cancer, resulting in increased chemoresistance and induction of AKT (71). Considering the important role AKT plays in stem cells, the suppression of this PP2A subunit could be important in supporting cancer stem cells. Furthermore, blockade of one of the PP2A scaffold subunits by a miR could be another means for suppression of PP2A function by a diverse number of PP2A isoforms. Supporting such a concept, recent work from the Perrotti group has demonstrated that activation of PP2A using FTY-720 in CML cells results in eradication of the leukemic stem cells thus the concept of PP2A regulation of stemness likely is pertinent to LSC as well (100). The role of miRs in stem cell differentiation is an active area of research but the field is just emerging. It remains to be determined if and how miRs and PP2A might interact to influence stem cell properties.

**B55α REGULATION OF miRs IN LEUKEMIA**

We recently identified a number of miRs that were subject to regulation by the B55α subunit that has been found to suppress AKT and PKCα survival signaling in AML cells (37, 38). The possible role of B55α in regulating miR expression was examined as we had shown the B subunit indirectly supported expression of MYC (38). MYC is known to control a number of miRs (47, 101–103). Suppression of B55α by shRNA in the AML cell line OCI-AML3 resulted in altered expression of a number of miRs. For the most part, reduction of the B subunit led to significant suppression (i.e., >2 fold) of over 30 miRs. This finding would be consistent with possible participation of MYC as MYC has been suggested to have a role in global repression of miRs (97). The miR most affected was miR-1260a, though not much is known about this miR. Among the other miRs that were suppressed are miR-142-3p, miR-142-5p, and miR-195-5p (38). The miR-142 members have been shown to be mutated in lymphoma and AML and thus are of interest to those studying hematologic malignancies (103, 104). Relevant for myeloid leukemia, miR-142-3p has been implicated in control of myeloid differentiation (83). With MYC reduction in the AML cells with reduced B55α, expression of the more prominent MYC targets including those in miR 17–92 cluster were not strongly...
FIGURE 2 | Model of feedback loop in B56α/AKT/MYC axis involving miRs. Model is presented where the B56α PP2A subunit and PTEN are negatively regulated by miR-155. The miR may act as a rheostat for AKT signaling as one the one hand PTEN suppression activates AKT but the miR also suppresses the B subunit which is positively regulated by AKT. The B subunit negatively regulates MYC and MYC supports expression of AKT targeting miR-29 so another layer of feedback may exist via MYC/miR-29.

FIGURE 3 | Model of effect of B55α/B56α competition on miR expression. Model is presented where the B55α and B56α PP2A subunits compete, resulting in additional regulation mediated by miRs. B55α supports miR-155 which targets B56α so an additional level of suppression exists in the competition between the two B subunits. B56α may have pro-survival function by suppressing B55α mediated expression of miR-191 and/or allowing expression of the miR-142 members, which are inhibited by B55α.
affected (38). However, induction of miR-195 observed in the cells could be mediated by MYC. MYC has been shown to regulate a number of miRs including miR-195 and it has been suggested that repression of anti-tumor miRs may be critical to MYC’s oncogenic activity (47). While it is not clear the role of miR-195 in leukemia, the miR has been implicated as a tumor suppressor in a number of solid tumor models (105). Targets of miR-195 include cyclins and cyclin dependent kinases (106), MYB (107), and NF κB signaling via IKKα and TAB3 (108). One of the miR-195 targets is the RET tyrosine kinase so perhaps inhibition of this kinase may be important in leukemia (109). Of the miRs elevated in response to suppression of B55α in the AML cells was miR-191-5p and miR-155 (38). The miR-191-5p has been implicated as being detrimental for patient survival in AML (89). As discussed earlier, a critical miR in leukemia and other cancers is miR-155. Interestingly, miR-155 has been shown to target B56α in macrophages (76). This finding and the possibility that B55α supports miR-155 expression suggests a complex regulatory mechanism whereby the B55 subunit can suppress the B56 subunit by competition for the catalytic core and by inducing a miR that targets that competing B subunit (depicted in Figure 3). This regulatory model is currently under investigation in our laboratory.

CONCLUSION

The role of miRs as regulators of PP2A and possible control of miR expression by PP2A isoforms is only just emerging. Publications covering miR control of PP2A subunits and regulators of PP2A (e.g., SET and CIP2A) are just emerging. Recent studies suggest that miR control of the PP2A genes is important as results indicate that dysregulation of PP2A targeting miRs can result in induction of signaling pathways associated with the particular PP2A isoform. An example of such a finding involves activation of AKT when miR-222 (which targets the B55α subunit) is elevated (72). The recent finding that miR-155 target genes include a PP2A subunit [i.e., B56α; Ref. (76)] raises the possibility that part of the tumor support properties of this miR may involve suppression of the B subunit. A link between miR-155 and B55α in leukemia cells remains to be established. Surprisingly, the regulation of miRs by PP2A has been less well studied. The well documented relationship between MYC and PP2A would suggest that there would be PP2A regulation of at least MYC sensitive miRs. Interestingly, though B55α serves to positively support MYC expression, there were no effects observed on MYC sensitive miRs in our recent study in AML (38). Still other miRs including miR-142, miR-191, and miR-155 appear to be controlled by the PP2A subunit (38). It remains to be seen how the B subunit regulates these miRs. Are specific transcription factors targeted or are effects mediated by suppression of kinases that phosphorylate transcription factors? Also, a role for other PP2A B subunits in miR regulation is presently unknown. It is likely that other B subunits will exert effects on miR expression and warrant investigation. A better understanding of how PP2A controls miRs and vice versa will provide a better understanding how survival signaling is dysregulated in leukemia and may lead to novel strategies for future therapies.

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REFERENCES

1. Janssens V, Longin S, Goris J. PP2A holoenzyme assembly: in cauda venenum (the sting is in the tail). Trends Biochem Sci (2008) 33:113–21. doi:10.1016/j.tibs.2007.12.004
2. Janssens V, Rebollo A. The role and therapeutic potential of Ser/Thr phosphatase PP2A in apoptotic signalling networks in human cancer cells. Curr Mol Med (2012) 12:268–87. doi:10.2174/15665241279218930
3. Murphy MC, Walter G. Protein serine/threonine phosphatases: structure, regulation, and function in cell growth. Physiol Rev (1993) 73:673–99.
4. Orsog S, Brewis ND, Alphey L, Duda Y, Cohen PT. The structure of protein phosphatase 2A is as highly conserved as that of protein phosphatase 1. FEBS Lett (1990) 275:44–6. doi:10.1016/0014-5793(90)81455-Q
5. McLellan B, Vinhshap DM. Identification of a new family of protein phosphatase 2A regulatory subunits. J Biol Chem (1995) 270:26123–8. doi:10.1074/jbc.270.44.26123
6. Stone SR, Hofsteenge J, Hemmings BA. Molecular cloning of cDNAs encoding two isoforms of the catalytic subunit of protein phosphatase 2A. Biochemistry (1987) 26:2151–20. doi:10.1021/bi00379a003
7. Hemmings BA, Adams-Pearson C, Maurer F, Muller F, Goris J, Merlevede W, et al. Alpha and beta forms of the 65k subunit of protein phosphatase 2A have similar 39 amino acid repeating structure. Biochemistry (1999) 28:1566–73. doi:10.1021/bi98065a002
8. Silverstein AM, Barrow CA, Davis AJ, Mumb CMC. Actions of PP2A on the MAP kinase pathway and apoptosis are mediated by distinct regulatory subunits. Proc Natl Acad Sci USA (2002) 99:4221–6. doi:10.1073/pnas.072071699
9. Strack S, Cribs JT, Gomez L. Critical role for protein phosphatase 2A hetrotrimers in mammalian cell survival. J Biol Chem (2004) 279:47732–9. doi:10.1074/jbc.M408015200
10. Ruvalco VR, Kurinna SM, Karanejeet KB, Schuster TF, Mertalli AM, McCabrey JA, et al. PKR regulates B56 (alpha)-mediated BCL2 phosphatase activity in acute human lymphoblastic leukemia-derived REH cells. J Biol Chem (2008) 283:35474–85. doi:10.1074/jbc.M800951200
11. Chen J, Parsons S, Brautigan DL. Tyrosine phosphorylation of protein phosphatase 2A in response to growth stimulation and v-src transformation of fibroblasts. J Biol Chem (1994) 269:7957–62.
12. Vacaru AM, Deng H, Kim S, Goris J. Interaction of simian virus 40 small tumor antigen with protein phosphatase 2A by controlling the association of regulatory B subunits. EMBO J (2000) 19:5682–91. doi:10.1093/emboj/19.21.5682
13. Trottay T, Lee J, Vafai S, Stock JB. Carboxyl methylation regulates phospho-protein phosphatase 2A by controlling the association of regulatory B subunits. J Biol Chem (2008) 283:39392–5. doi:10.1074/jbc.M800951200
14. Yang SI, Lickteig RL, Estes R, Rundell K, Walter G, Mumby MC. Control of protein phosphatase 2A by simian virus 40 small-T antigen. Mol Cell Biol (1991) 11:9888–95.
15. Mateer SC, Fedorov SA, Mumb CMC. Identification of structural elements involved in the interaction of simian virus 40 small tumor antigen with protein phosphatase 2A. J Biol Chem (1998) 273:35339–46. doi:10.1074/jbc.273.52.35339
16. Mullan KE, Rawnfock M, Muller C, Schaffhausen B. Signaling from polyoma small and middle T antigens and SV40 small T antigen form stable complexes with protein phosphatase 2A. Cell (1990) 60:167–76. doi:10.1016/0092-8674(90)90726-U
17. Vacaru AM, Deng H, Kim S, Goris J. Interaction of simian virus 40 small tumor antigen with protein phosphatase 2A by controlling the association of regulatory B subunits. EMBO J (2000) 19:5682–91. doi:10.1093/emboj/19.21.5682
18. Pallas DC, Shahrak IK, Martin BL, Jaspers S, Miller TB, Brautigan DL, et al. Polyma small and middle T antigens and SV40 small T antigen form stable complexes with protein phosphatase 2A. Cell (1990) 60:167–76. doi:10.1016/0092-8674(90)90726-U
19. Frey D, Nevinian N, ReSetting PP2A tumour suppressor activity in blast crisis chronic myelogenous leukemia. Br J Cancer (2006) 95:775–81. doi:10.1038/sj.bjc.6603317
20. Nevinian N, Pantham N, Oakes J, Eisen M, Nevinian N, Blaser BW. FT720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. J Clin Invest (2007) 117:2408–21. doi:10.1172/JCI31095
21. Yang Y, Huang Q, Lu Y, Li X, Huang S. Reactivating PP2A by FT7720 as a novel therapy for AML with C-Kit tyrosine kinase domain mutation. J Cell Biochem (2011) 112:1314–22. doi:10.1002/jcb.23003

22. Perrotti D, Neviani P. Protein phosphatase 2A (PP2A), a druggable tumor suppressor in Ph(+) leukemias. Cancer Metastasis Rev (2008) 27:159–68. doi:10.1007/s10555-008-9119-x

23. Perrotti D, Neviani P. Protein phosphatase 2A: a target for anticancer therapy. Lancet Oncol (2013) 14:e229–38. doi:10.1016/S1474-4153(12)70558-2

24. Calin GA, Pekarsky Y, Croce CM. Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms in human neoplasms. Oncogene (2000) 19:1191–5. doi:10.1038/sj.onc.1203389

25. Wang SS, Esplin ED, Li JL, Huang L, Gazdar A, Minna J, et al. Alterations of protein phosphatase 2A regulatory subunit B55 alpha activator, and microRNA expression in acute myeloid leukemia cells. Proc Natl Acad Sci U S A (2009) 106:12974–9. doi:10.1073/pnas.0811267106

26. Wei S, Chen X, Rocha K, Epling-Burnette PK, Djeu JY, Liu Q, et al. A critical role for phosphatase haplodeficiency in the selective suppression of deletion 5q MDS by lenalidomide. Proc Natl Acad Sci U S A (2010) 107:206–11. doi:10.1073/pnas.0909704107

27. Giagounidis A, Mufti GJ, Fenaux P, Germing U, List A, Macbeth KJ. Lenalidomide as a disease-modifying agent in patients with del(5q) myelodysplastic syndromes: linking mechanism of action to clinical outcomes. Ann Hematol (2014) 93:111–14. doi:10.1007/s00277-013-1863-5

28. Kornblau SM, Womble M, Qiu YH, Jackson CE, Chen W, Konopleva M, et al. Simultaneous activation of multiple signal transduction pathways confers poor prognosis in acute myeologenous leukemia. Blood (2006) 108:2358–65. doi:10.1182/blood-2006-02-034735

29. Scholl C, Gilliland DG, Freihofer S. Deregulation of signaling pathways in acute myeloid leukemia. Semin Oncol (2008) 35:336–45. doi:10.1053/j.seminoncol.2008.04.004

30. Martelli AM, Evangelisti C, Chiarioni F, McCubrey JA. The phosphatidylinositol 3-kinase/Akt/mTOR signaling network as a therapeutic target in acute myeologenous leukemia patients. Oncotarget (2010) 1:89–103.

31. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Montalto G, Cervello M, et al. Mutations and deregulation of Ras/Raf/MEK/ERK and PI3K/AKT/PTEN/mTOR cascades. Oncotarget (2012) 3:954–87.

32. Welch JS, Ley TJ, Link DC, Miller GA, Larson DE, Koboldt DC, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell (2012) 150:264–78. doi:10.1016/j.cell.2012.06.023

33. Gallay N, Dos Santos C, Cuzin L, Bousquet M, Simonnet Gouy V, Chausse C, et al. The level of AKT phosphorylation on threonine 308 but not on serine 473 is associated with high-risk cytogenetics and predicts poor prognosis in acute myeloid leukemia. Leuk Lymphoma (2009) 50:12974–9. doi:10.1080/10428190802648387

34. Cristóbal I, Cirauqui C, Castello-Cros R, Garcia-Orti L, Calasanz MJ, Odero MD, et al. SETBP1 overexpression is a novel leukemogenic mechanism that predicts poor prognosis in acute myelogenous leukemia. Proc Natl Acad Sci U S A (2002) 99:15324–9. doi:10.1073/pnas.242606799

35. Kuney T, Godnic I, Horvat S, Zorc M, Calin GA. Cross talk between microRNA and coding cancer genes. Cancer J (2012) 18:223–31. doi:10.1097/PPO.0b013e3182587f71

36. Van Roosbroek K, Pollet J, Calin GA. miRNAs and long noncoding RNAs as biomarkers in human diseases. Expert Rev Mol Diagn (2013) 13:183–204. doi:10.1586/erm.12.134

37. Curtan AM, Sharp PA. The role of miRNAs in regulating gene expression networks. J Mol Biol (2013) 425:3582–600. doi:10.1016/j.jmb.2013.03.007

38. Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, et al. Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet (2008) 40:43–50. doi:10.1038/ng.2007.30

39. Copley MR, Eaves CJ. Developmental changes in hematopoietic stem cell properties. Exp Mol Med (2013) 45:e55. doi:10.1038/emm.2013.88

40. Shy-b-Chang N, Daley QG. Lin28: primal regulator of growth and metabolism in stem cells. Cell Stem Cell (2012) 12:395–406. doi:10.1016/j.stem.2013.03.005

41. Chen Y, Jacamo R, Konopleva M, Garzon R, Croce C, Andreff M. CXCR4 downregulation of let-7a drives chemoresistance in acute myeloid leukemia. J Clin Invest (2013) 123:239–407. doi:10.1172/JCI66553

42. Calin GA, Croce CM. MicroRNAs and chromosomal abnormalities in cancer cells. Oncogene (2006) 25:6202–10. doi:10.1038/sj.onc.1209910

43. Garzon R, Fabbri M, Cinimin A, Calin GA, Croce CM. MicroRNA expression and function in cancer. Trends Mol Med (2006) 12:580–7. doi:10.1016/j.molmed.2006.10.006

44. Marcucci G, Radmacher MD, Mrozek K, Bloomfield CD. MicroRNA expression in acute myeloid leukemia. Curr Hematol Malig Rep (2009) 4:83–8. doi:10.1007/s11899-009-0012-7

45. Aquelín RI, Calin GA, Croce CM. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. Cell Death Differ (2010) 17:215–20. doi:10.1038/cdd.2009.69

46. Calin GA, Pekarsky Y, Croce CM. The role of microRNA and other non-coding RNA in the pathogenesis of chronic lymphocytic leukemia. Best Pract Res Clin Haematol (2007) 20:425–37. doi:10.1016/j.beha.2007.02.003

47. Wang Y, Li Z, He C, Wang D, Yuan X, Chen J, et al. MicroRNA expression signatures are associated with lineage and survival in acute leukemias. Blood Cells Mol Dis (2010) 44:191–7. doi:10.1016/j.bcmd.2009.12.010

48. Barbarotto E, Calin GA. Potential therapeutic applications of microRNA-based technology in hematological malignancies. Curr Pharm Des (2008) 14:2040–50. doi:10.2174/138945008782549627

49. Pons A, Nomdedeu B, Navarro A, Gaya A, Gel B, Diaz T, et al. Hematopoiesis-related microRNA expression in myelodysplastic syndromes. Leuk Lymphoma (2009) 50:1854–9. doi:10.1080/10428190903147643

50. O’Connell RM, Chaudhuri AA, Rao DS, Baltimore D. Insoluble phosphatase SHP1 is a primary target of miR-155. Proc Natl Acad Sci U S A (2009) 106:7113–8. doi:10.1073/pnas.0902636106

51. Rocah OH, Granot G, Ovcharenko A, Modai S, Pasmanik-Chor M, Toren A, et al. Downregulation of miR-31, miR-155, and miR-584 in chronic myeloid leukemia cells. PloS One (2012) 7:e35501. doi:10.1371/journal.pone.0035501

52. Metzeler KH, Makarovsky K, Kolbischmidt J, Volinia S, Mrózek K, Becker H, et al. Astem cell-like gene expression signature associates with inferior outcomes and a distinct microRNA expression profile in adults with primary cytogenetically normal acute myeloid leukemia. Leukemia (2013) 27:2023–31. doi:10.1038/leu.2013.181

53. Marcucci G, Makarovsky KS, Metzeler KH, Volinia S, Wu YZ, Mrózek K, et al. Clinical role of microRNAs in cytogenetically normal acute myeloid leukemia.
miR-155 upregulation independently identifies high-risk patients. J Clin Oncol (2013) 31:2086–93. doi:10.1200/JCO.2012.45.6228
63. Cui B, Chen L, Zhang S, Mraz M, Fecteau JF, Yu J, et al. MicroRNA-155 influences B-cell receptor signaling and associates with aggressive disease in chronic lymphocytic leukemia. Blood (2014) 124:546–54. doi:10.1182/blood-2014-03-559690
64. Li X, Zhang J, Gao L, McClellan S, Finan MA, Butler TW, et al. miR-181 mediates cell differentiation by interrupting the Lin28 and let-7 feedback circuit. Cell Death Differ (2012) 19:378–86. doi:10.1038/cdd.2011.127
65. Li Z, Huang H, Li Y, Jiang X, Chen P, Aronovitz S, et al. Up-regulation of a HOXA-PBX3 homeobox-gene signature following down-regulation of miR-181 is associated with adverse prognosis in patients with cytogenetically abnormal AML. Blood (2012) 119:2314–24. doi:10.1182/blood-2011-10-386235
66. Chen HG, Han WJ, Deng M, Qin J, Yuan D, Liu JP, et al. Transcriptional regulation of PP2A-A alpha is mediated by multiple factors including AP-2alpha, CREB, ETS-1, and SP-1. PLoS One (2009) 4:e7019. doi:10.1371/journal.pone.0007019
67. Chen HF, Mai JR, Wan JX, Gao YF, Lin LN, Wang SZ, et al. Role of a novel transcription factor Ikaros in the positive regulation of c-Myc and other key oncoproteins. J Biol Chem (2010) 285:5067–108. doi:10.1074/jbc.M108000452.CAN-09-4148
68. Nagpal K, Watanabe KS, Tsao BP, Tsokos GC. Transcription factor Ikaros enhances the expression of the oncogenic miR-222 in lung cancer cells. Leukemia (2009) 23:3029–38. doi:10.1038/onc.2013.157
69. Su JL, Chen PB, Chen YH, Chen SC, Chang YW, Jan YH, et al. Downregulation of miR-520h-5p by E1A contributes to anticancer activity. Mol Cancer Res (2010) 8:867–75. doi:10.1158/1541-7786.MCR-09-1840
70. Yu YH, Chen HA, Chen PS, Cheng YJ, Hsu WH, Chang YW, et al. miR-520h-mediated FOXC2 regulation is critical for inhibition of lung cancer progression by resveratrol. Oncogene (2013) 32:431–43. doi:10.1038/onc.2012.74
71. Hamano R, Miyata H, Yamazaki M, Kurokawa Y, Harai M, Moon JH, et al. Over-expression of miR-20c induces chemoresistance in esophageal cancers mediated through activation of the Akt signaling pathway. Clin Cancer Res (2011) 17:3029–38. doi:10.1158/1078-0432.CCR-10-2532
72. Wong QW, Ching AK, Chan AW, Choy KW, To KF, Lai PB, et al. miR-31 functions as an oncogenic microRNA in mouse and human lung cancer cells targeting patterns in the differentiation of human embryonic stem cells. Stem Cells (2010) 28:1874–84. doi:10.1002/stem.412
73. Aronov HD, Sears RC. A tumor suppressor role for PP2A-B56alpha through negative regulation of c-Myc and other key oncoproteins. Cancer Metasasis Rev (2008) 27:147–58. doi:10.1007/s10555-008-9128-9
74. Jurkovicova D, Magyerkova M, Kulcsar L, Krivjanska M, Krivjansky V, Gibadullinova A, et al. miR-135 as a diagnostic and prognostic marker in hematological and solid malignancies. Natl Inst Med Sci U S A (2006) 103:5078–83. doi:10.1038/ncms00587103
75. Undi RB, Kandi R, Gutt RK. MicroRNAs as haematopoiesis regulators. Adv Hematol (2013) 2013:697534. doi:10.1155/2013/697534
76. Ferracin M, Zagatti B, Rizzato L, Cavazini F, Veronese A, Cicoone M, et al. MicroRNAs involvement in thalassemia refractory chronic lymphocytic leukemia. Mol Cancer (2010) 9:123. doi:10.1186/1476-4598-9-123
77. Feng X, Wang Z, Fillmore R, Xi Y. miR-200, a new star miRNA in human cancer. Cancer Biol Med (2010) 7:208–16. doi:10.3892/mb.2010.123
78. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. Nature (2004) 435:828–33. doi:10.1038/nature03552
79. Feng X, Wang Z, Fillmore R, Xi Y. miR-200, a new star miRNA in human cancer. Cancer Biol Med (2010) 7:208–16. doi:10.3892/mb.2010.123
80. Suzuki HI, Matsuyama H, Noguchi M, Yao T, Komatsu N, Mano H, et al. Computational dissection of distinct microRNA activity signatures associated with peripheral T cell lymphoma subtypes. Leukemia (2013) 27:2107–11. doi:10.1038/leu.2013.121
81. Chao A, Tsai CL, Wei PC, Hsheu S, Chao AS, Wang CJ, et al. Decreased expression of microRNA-199b increases protein levels of SET (protein phosphatase 2A inhibitor) in human chorionicarcinoma. Cancer Lett (2010) 291:89–97. doi:10.1016/j.canlet.2009.10.005
82. Jung HM, Patel RS, Phillips BL, Wang H, Cohen DM, Reinhold WC, et al. Tumor suppressor miR-375 regulates MYC expression via repression of CIP2A coding sequence through multiple microRNA-mRNA interactions. Mol Biol Cell (2013) 24:1638–1648:S1–7. doi:10.1091/mbc.E12-12-0891
83. Wang XS, Gong JN, Yu J, Wang Y, Zhang XH, Tian ZQ, et al. MicroRNA-29a and microRNA-142-3p are regulators of myeloid differentiation and acute myeloid leukemia. Blood (2012) 119:4992–5004. doi:10.1182/blood-2011-10-385716
84. Forrest AR, Kanamori-Katayama M, Tomaru Y, Lasmann T, Ninomiya T, Taka-hashi Y, et al. Induction of microRNAs, miR-155, miR-222, miR-424 and miR-503, promotes monocytic differentiation through combinatorial regulation. Leukemia (2010) 24:460–6. doi:10.1038/leu.2009.246
85. Karashishi P, Palombo T, Iuliano R, Cinmino A, Aquilan R, et al. MicroRNA fingerprints during human megakaryocytogenesis. Proc Natl Acad Sci U S A (2006) 103:5078–83. doi:10.1073/pnas.0600587103
86. Undi RB, Kandi R, Gutt RK. MicroRNAs as haematopoiesis regulators. Adv Hematol (2013) 2013:697534. doi:10.1155/2013/697534
87. Ferracin M, Zagatti B, Rizzato L, Cavazini F, Veronese A, Cicoone M, et al. MicroRNAs involvement in thalassemia refractory chronic lymphocytic leukemia. Mol Cancer (2010) 9:123. doi:10.1186/1476-4598-9-123
88. Feng X, Wang Z, Fillmore R, Xi Y. miR-200, a new star miRNA in human cancer. Cancer Biol Med (2010) 7:208–16. doi:10.3892/mb.2010.123
89. Suzuki HI, Matsuyama H, Noguchi M, Yao T, Komatsu N, Mano H, et al. Computational dissection of distinct microRNA activity signatures associated with peripheral T cell lymphoma subtypes. Leukemia (2013) 27:2107–11. doi:10.1038/leu.2013.121
102. Aguda BD, Kim Y, Piper-Hunter MG, Friedman A, Marsh CB. MicroRNA regulation of a cancer network: consequences of the feedback loops involving miR-17-92, E2F, and Myc. Proc Natl Acad Sci U S A (2008) 105:19678–83. doi:10.1073/pnas.081166106

103. Kwanhian W, Lenze D, Alles J, Motsch N, Barth S, Doll C, et al. MicroRNA-142 is mutated in about 20% of diffuse large B-cell lymphoma. Cancer Med (2012) 1:141–55. doi:10.1002/cam4.29

104. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med (2013) 368:2059–74. doi:10.1056/NEJMoa1301689

105. Finnerty JR, Wang WX, Hébert SS, Wilfred BR, Mao G, Nelson PT. The miR-15/107 group of microRNA genes: evolutionary biology, cellular functions, and roles in human diseases. J Mol Biol (2010) 402:491–509. doi:10.1016/j.jmb.2010.07.051

106. Xu T, Zha Y, Xiong Y, Ge YY, Yun JP, Zhuang SM. MicroRNA-195 suppresses tumorigenicity and regulates G1/S transition of human hepatocellular carcinoma cells. Hepatology (2009) 50:113–21. doi:10.1002/hep.22919

107. Yongchun Z, Linwei T, Xicai W, Lianhua Y, Guangqiang Z, Ming Y, et al. MicroRNA-195 inhibits non-small cell lung cancer cell proliferation, migration and invasion by targeting MT1B. Cancer Lett (2014) 347:65–74. doi:10.1016/j.canlet.2014.01.019

108. Ding J, Huang S, Wang Y, Tian Q, Zha R, Shi H, et al. Genome-wide screening reveals that miR-195 targets the TNF-α/NF-κB pathway by down-regulating 1κB kinase alpha and TAB3 in hepatocellular carcinoma. Hepatology (2013) 58:654–66. doi:10.1002/hep.26378

109. Díaz-Beyá M, Navarro A, Ferrer G, Díaz T, Gel B, Camós M, et al. Acute myeloid leukemia with translocation (8;16)(p11;p13) and MYST3-CREBBP rearrangement harbors a distinctive microRNA signature targeting RET proto-oncogene. Leukemia (2013) 27:595–603. doi:10.1038/leu.2012.278

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