Published in final edited form as:

Cell Mol Life Sci. 2012 November ; 69(21): 3601–3612. doi:10.1007/s00018-012-1129-8.

The Akt-associated microRNAs

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Abstract

As master gene regulators, microRNAs impact diverse cellular pathways. It is well known that microRNAs are often dysregulated in many types of cancer and other human diseases. In cancer, microRNAs may function as oncogenes or tumor suppressors. Of considerable interest, recent evidence suggests that microRNA-mediated gene regulation interconnects with the Akt pathway, forming an Akt-microRNA regulatory network. MicroRNAs and Akt in this network work together to exert their cellular functions. Thus, a better understanding of this Akt-microRNA regulatory network is critical to successful targeting of the PI3K/Akt pathway for cancer therapy. In this review, we will update on recent advances of how microRNAs impact the Akt activity as well as how microRNAs are regulated through the Akt pathway. We will also briefly discuss the clinical implication of gene regulation mediated through the Akt-associated microRNAs.

Introduction

The serine/threonine kinase (Akt)/protein kinase B (PKB) is a key downstream target of the phosphoinositide-3 kinase (PI3K)-mediated signaling pathway and it plays an important role in regulation of diverse cellular processes. Inappropriate activation of Akt has been reported in many types of human diseases, in particular cancer. As a major pathway involved in cell growth and proliferation, the Akt pathway has been intensively investigated. Recent evidence suggests that newly discovered microRNAs are important molecular players in this pathway, thus adding another layer of complexity. For the sake of simplicity, we will focus on two groups of microRNAs, i.e., those upstream or downstream Akt. MicroRNAs at the upstream are those capable of regulating Akt activity, whereas microRNAs at the downstream often serve as Akt effectors. Apparently, these microRNAs and Akt work together to exert their cellular functions. Therefore, any new information on these microRNAs will add in our better understanding of this Akt-microRNA regulatory network and help develop better strategies for cancer treatment. We will first discuss how microRNAs regulate gene expression, and their function in general. We will then devote our major effort to discussions of these two groups of microRNAs, and their roles in gene expression and tumorigenesis. We will take miR-145 and miR-101 as examples for these two groups of microRNAs.

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MicroRNAs as master gene regulators

MicroRNAs are a class of naturally occurring small non-coding RNAs that are capable of regulating expression of protein-coding genes at the posttranscriptional level (1). Evidence indicates that microRNAs exert their silencing function usually by interactions with the 3'-untranslated region (3'-UTR) of a target gene through imperfect base-pairing. Because of this unique feature, a single microRNA can have multiple targets and thus, microRNAs could regulate a large number of protein-coding genes (2, 3). In particular, many of these targets are involved in various signaling pathways so that their impact on gene expression can be significantly amplified. Therefore, microRNAs are master gene regulators, similar to transcription factors. Of great interest, microRNAs and transcription factors may cooperate and ultimately determine gene expression patterns in the cell (4). Given that microRNAs are able to directly impact both mRNA (degradation) and protein levels (translation repression), they could play a more important role than transcription factors to certain extent, because transcription factors are supposed to regulate RNA transcription alone. Importantly, by serving a fine tuning role, microRNAs may be able to make a final decision as to whether a given gene is turned up or down (5). Therefore, a better understanding of how microRNAs regulate gene expression, especially in the context of pathway specific manner, will greatly facilitate biomarker discovery and help design new strategies for cancer treatment.

Since microRNAs can target multiple genes, it is not surprising that they impact a variety of cellular pathways. Early studies have shown that microRNAs are critical to developmental timing, cell death, cell proliferation and patterning of the nervous system. Now it becomes evident that microRNAs may play a much broader role in regulation of cellular functions; dysregulation of microRNA expression could lead to a variety of human disorders, especially cancer. In this regard, microRNAs may function as oncogenes or tumor suppressors (6).

Given that microRNAs are frequently dysregulated in cancer, an important question is how microRNAs are regulated. Like protein-coding genes, pri-microRNA transcripts can be generated by polymerase II (Pol II) (7). Evidence indicates that the regulation of microRNA expression is very similar to that of protein-coding gene expression. For instance, transcription factors such as c-Myc and p53 could play a key role in regulation of microRNA expression. The miR-17~92 cluster has been shown to be induced by c-Myc (8, 9). The p53 status can also affect expression of many microRNAs in colon cancer cells, leading to differential expression of many potential microRNA target genes (10). Moreover, p53 is a key regulator for expression of the miR-34 family including miR-34a, miR-34b and miR-34c (11). Similarly, we have demonstrated that miR-145 is a direct transcription target for p53 through interaction with the miR-145 promoter (12). On the other hand, transcriptional repressors could negatively regulate microRNA expression. For example, REST suppresses miR-21 to maintain stemness (13). In addition to transcriptional regulation, epigenetic factors, such as acetylation and methylation (14, 15) or factors involved in microRNA biogenesis or processing (16–19), may also play a role in regulating microRNA expression.

These new findings clearly indicate that just like protein-coding genes, microRNA expression is under complex regulation. New evidence further indicates that these regulatory systems can be categorized into different pathways such as Akt. Therefore, this review will focus on recent advancements on how Akt impacts microRNA expression as well as how microRNAs regulate the Akt pathway.

Function of microRNAs

Mounting evidence suggests that microRNAs play a very broad role in a variety of cellular pathways, including development, differentiation, cell proliferation, and apoptosis. Given the potentially large number of genes that can be regulated by microRNAs, they may affect many aspects of every cellular pathway. We list here a few examples which are closely related to the current topic, including development/differentiation, cell proliferation/apoptosis, and pluripotency/stem cells.

To date, a large number of microRNAs have been shown to play a role in development and cell differentiation. The functional importance of microRNAs in development can be deputized from animals that lack the microRNA processing enzyme Dicer, and these animals show early arrest in development accompanied by defects in proliferation of stem cells (20). Similarly, mice deficient in miR-17~92 cluster die shortly after birth with lung hypoplasia (21). These findings highlight the role of these microRNAs as developmental switches.

Some microRNAs are specifically expressed in hematopoietic cells and their expression is dynamically regulated during early hematopoiesis and lineage commitment. For example, an early study showed that miR-181 is preferentially expressed in the B-lymphoid cells of mouse bone marrow, and its ectopic expression in hematopoietic stem/progenitor cells leads to an increased fraction of B-lineage cells in both tissue-culture differentiation assays and adult mice (22). In another case, microRNA expression is associated with TPA-induced differentiation of human leukemia cells (HL-60) into monocyte/macrophage-like cells (23). Similarly, miR-143 seems to play a role in adipocyte differentiation because miR-143 levels increase in differentiating adipocytes, and inhibition of miR-143 effectively inhibits adipocyte differentiation (24). MicroRNAs have also been shown to regulate muscle differentiation where miR-1 may function as a regulator for “quality control” during muscle development by blocking detrimental mRNAs that are promiscuously expressed (25). Moreover, it has been estimated that 71% of the differentially expressed genes in 3T3-L1 cells during differentiation could be regulated by microRNAs (26). Of interest, miR-133 specifically targets the neuronal homolog of polypyrimidine tract-binding protein (nPTB), a splicing factor, which is responsible for myoblast differentiation (27). Furthermore, the neuronal repressor REST maintains self-renewal and pluripotency in mouse ES cells through suppression of miR-21 (13), suggesting the role of miR-21 as a differentiation factor.

Given that the aberrant expression of microRNAs is associated with many types of cancer, it is believed that microRNAs influence cell proliferation and apoptosis. Indeed, there are an overwhelming number of microRNAs associated with these functions. For example, miR-34 family is positively regulated by p53 (28). Overexpression of miR-34a promotes p53-mediated apoptosis (29, 30). On the other hand, miR-21 exerts an anti-apoptotic function because suppression of miR-21 leads to increased apoptosis (31, 32). We will not discuss details due to space limit.

Isolation of a number of stem cell-specific microRNAs implicates their role in controlling stem cell division (33). An early work by Hatfield et al demonstrated that the microRNA pathway is required for proper control of germline stem cell (GSC) division in *Drosophila* (34). Using GSC mutants for dicer-1 (*dcr-1*), this group was able to show that these mutants are defective in cell cycle control (34). Thus, microRNAs may be involved in the mechanism that makes stem cells insensitive to environmental stimuli that would normally halt most cells at the G₁/S checkpoint. Several stem cell-specific microRNAs have been identified in humans and mice (35, 36). The majority of these stem cell-specific microRNAs

are only expressed in undifferentiated stem cells; their expression is significantly reduced as embryonic stem cells differentiate into embryoid bodies and is undetectable in adult organs.

It is known that certain cancer cells have similar properties to stem cells, i.e., both can avoid cell division stop signals and keep dividing to form new cells. Since microRNAs are required to bypass the normal G₁/S checkpoint for appropriate stem cell renewal and loss-of-function of these microRNAs reduces the proliferation of stem cells (34), stem cell-specific microRNAs may have direct implications in the development of cancer. In particular, apoptosis and cell proliferation pathways are frequently altered in tumor cells and thus, microRNAs could play a causal role in tumor initiation and progression by regulating these pathways.

Clinical significance of microRNAs

The clinical implication of microRNAs lies in the findings that dysregulation of microRNAs affects normal cell growth and development, leading to a variety of disorders including neurological diseases (37) and human cancer (38–42). In cancer, specific overexpression or underexpression has been shown to correlate with particular tumor types because overexpression of a particular set of microRNAs could result in down-regulation of tumor suppressor genes, whereas their underexpression could lead to oncogene up-regulation (6). Since microRNAs are often located at fragile sites or in repetitive genomic sequences of chromosomal regions (43), this may explain why dysregulation of microRNA expression occurs frequently in human cancer. For instance, 68% of investigated patients suffering from B-cell chronic lymphocytic leukemia (CLL) have been shown to have a deletion located at chromosome 13q14 where the miR-15 and miR-16 genes reside and are under-represented in many B-CLL patients (44).

Apparently, whether microRNA functions as an oncogene or tumor suppressor is largely determined by the target genes of each particular microRNA. For example, tumor suppressive microRNAs, such as let-7, miR-15 and miR-16, are able to suppress expression of oncogenes. let-7 suppresses Ras oncogene and is downregulated in lung cancer (45); miR-15 and miR-16 suppress Bcl-2 anti-apoptotic gene, and they are deleted or downregulated in leukemia (44, 46). In contrast, oncogenic microRNAs can silence tumor suppressor genes. miR-17-5p and miR-20a control the balance of cell death and proliferation driven by the proto-oncogene c-Myc (9) and miR-17-5p serves as an oncogene in lymphoma and lung cancer (8, 47). Similarly, a cluster consisting of miR-372 and miR-373 have been shown to function as oncogenes in testicular germ cell tumors by suppressing the p53 pathway (48). We and others have demonstrated that antisense miR-21 oligonucleotide suppresses tumor cell growth which is associated with increased apoptosis and decreased cell proliferation (31, 49), suggesting that miR-21 is an oncogene. We subsequently identified the tumor suppressor gene tropomyosin 1 (TPM1) as a direct miR-21 target gene (50). Furthermore, miR-21 also plays a role in cell invasion and tumor metastasis, which is likely through regulation of multiple miR-21 target genes, such as TPM1, programmed cell death 4 (pdc4) and maspin (51). Of interest, certain microRNAs may specifically modulate only tumor metastasis. For example, miR-10b functions as a metastasis initiation factor and overexpression of miR-10b causes breast tumor invasion and metastasis, but it has no effect on primary tumor growth (52). On the other hand, miR-335 suppresses metastasis and migration through targeting of the progenitor cell transcription factor SOX4 and extracellular matrix component tenascin C (53). Therefore, many of these microRNAs may serve as therapeutic targets.

Importantly, a unique set of microRNAs (or microRNA signatures) are often associated with human cancer. Lu et al reported a general downregulation of a number of microRNAs in

tumors compared with normal tissues in multiple human cancers (54). In particular, microRNA expression profiles are able to successfully classify poorly differentiated tumors whereas mRNA profiles are highly inaccurate for the same samples (54). MicroRNA signatures have also been reported in other types of cancers, including chronic lymphocytic leukemia (CLL) (55), lung cancer (56), pituitary adenomas (57), uterine leiomyomas (58) and adult acute myeloid leukemia (AML) (59). In lung cancer, microRNA expression profiles correlate with survival of lung adenocarcinomas, including those classified as disease stage I; high miR-155 and low let-7a-2 expression correlates with poor survival (56). Hierarchical clustering analysis of microRNA expression profiles is able to distinguish tumor from normal pancreas, pancreatitis and cell lines (60). In pituitary adenomas, 30 microRNAs are differentially expressed between normal pituitary and pituitary adenomas and among them, 24 microRNAs can serve as a predictive signature of pituitary adenoma and 29 microRNAs are able to predict pituitary adenoma histotype (57). In human uterine leiomyomas, 31 of 206 microRNAs examined reveal a distinct microRNA expression profile associated with tumor size and race (58). Furthermore, a solid cancer microRNA signature is suggested by a large portion of overexpressed microRNAs from a large-scale miRnome analysis on 540 samples, including lung, breast, stomach, prostate, colon, and pancreatic tumors (61). Together, these findings highlight the potential of microRNA profiling in cancer diagnosis (54).

The Akt-microRNA regulatory network

The role of Akt in cancer is well established and thus, targeting the Akt pathway for cancer therapy has been intensively investigated in recent years (62). Similarly, microRNAs can also play an important role in cancer initiation and progression as described above. Despite overwhelming preclinical trials suggesting that the Akt pathway is a good therapeutic target, clinical trials have shown the limited efficacy of single-agent of Akt pathway inhibitors (63), presumably due to the presence of signaling feedback loops in cells. Inhibition of Akt might alleviate the repression of other pro-survival and growth pathways. However, it is not fully understood as to what molecular players contribute to these survival and growth pathways. Currently, there is still a lack of reliable biomarkers that can precisely predict drug responses. Although several molecular biomarkers have been currently used in clinic, such as phosphorylated Akt and phosphorylated S6K1, these markers often reveal great variability and poor reproducibility. Thus, identification of new and potentially more robust biomarkers is critical to the preclinical development of Akt inhibitors (62). In this regard, these Akt-associated microRNAs may potentially serve as such biomarkers and moreover, they may also prove to be novel therapeutic targets. Accordingly, a better understanding of Akt-associated microRNAs will lead to the improvement of the efficacy of targeting Akt pathway.

Akt plays a key role in the cell in response to growth factors; it controls cell survival, cell cycle progression, metabolism and angiogenesis. Several known factors control Akt activity positively or negatively; a notable negative regulator is the tumor suppressor PTEN (Fig. 1). Of interest, PTEN can be targeted by a number of microRNAs directly and indirectly, leading to Akt activation. In addition, the protein phosphatase family such as PP2A (64) function as negative regulators because as phosphatases, they inactivate Akt by removing the phosphate group. On the other hand, the carboxyl-terminal modulator protein (CTMP) binds specifically to the carboxyl-terminal regulatory domain of Akt, and such binding reduces the Akt activity by inhibiting phosphorylation on serine 473 and threonine 308 (65). Therefore, microRNAs capable of targeting these negative regulators would also increase the Akt activity. At the downstream of Akt, a large number of Akt effectors have been identified. Notable examples are the forkhead transcription factors, the Foxo family. Once phosphorylated by Akt, the Foxo protein is translocated out of the nucleus and then

degraded through ubiquitin proteasome pathway (66). Similarly, Akt can phosphorylate MDM2, which enhances its nuclear localization; subsequently, the phosphorylated MDM2 binds to p53 and causes degradation of p53 by ubiquitin-proteasome pathway (67, 68). Unlike PTEN, both the Foxo and p53 are transcription factors. While they are under control of Akt, they are themselves targets for microRNAs. In particular, a large number of microRNAs are known to be regulated by either Foxo or p53. Therefore, alterations of the Akt level or activity will ultimately impact these transcription factors as well as corresponding microRNAs (Fig. 1).

MicroRNAs as Akt effectors

Although Akt controls multiple downstream targets or pathways, we will discuss two key downstream effectors Foxo and p53.

Foxo

As a direct target of Akt, Foxo can be regulated by microRNAs which are often upregulated in cancer. For instance, miR-182 is commonly upregulated in human melanoma; ectopic expression of miR-182 enhances migration of melanoma cells in vitro and their metastatic potential in vivo; in contrast, suppression of miR-182 inhibits invasion and promotes apoptosis (69). This is mainly due to the ability of miR-182 to target Foxo3a and microphthalmia-associated transcription factor (69). In addition, several microRNAs such as miR-155 and miR-96 have also been shown to target Foxo3a. For instance, miR-155 directly targets Foxo3a in breast cancer so that it regulates cell survival, growth, and chemosensitivity (70). Similarly, miR-96 is capable of silencing Foxo3a through its interactions with the 3'-UTR (71).

Since Foxo is a transcription factor, it can regulate protein-coding genes as well as noncoding genes including microRNAs. There are several examples where Foxo can either upregulate or down regulate microRNA expression. For instance, miR-21 is a well-known oncogenic microRNA (31, 32, 49, 51, 72). Several transcription factors can transcriptionally regulate its expression. Notably, Foxo3a serves as a negative regulator of miR-21 (73). Knockdown of Foxo3a resulted in an elevation in miR-21 levels, whereas enforced expression of Foxo3a led to a decrease in miR-21 expression (73). This is likely through direct interaction with the miR-21 promoter. On the other hand, both Foxo1 and Foxo3a positively regulate miR-145 through which miR-145 serves as a key component of Foxo-Mxi1-SRA/miR-145 axis as a major progression block in renal tumor development (74). Similarly, Foxo3a is able to increase the level of miR-17 such that it is involved in the Vitamin D(3) up-regulated protein 1 (VDUP1)-mediated cell senescence (75). However, since miR-17 is within the miR-17~92 cluster, how exactly Foxo3a regulates miR-17 and what the effect on other microRNAs in this cluster remains to be determined yet.

p53

Another important Akt target is MDM2 which negatively regulates the p53 activity. Phosphorylation of MDM2 by Akt leads to the nuclear localization of MDM2 so that MDM2 is able to bind to p53 and causes p53 degradation (76). As a key transcription factor, p53 has been shown to induce a large number of microRNAs including miR-34 family and miR-145. For example, we have demonstrated that miR-145 play a critical role in p53-mediated c-Myc repression, which may provide a possible explanation for the negative relationship between p53 and c-Myc. Of interest, p53 also regulates miR-145 through a posttranscriptional regulation mechanism (77).

A good example for p53-mediated regulation is the miR-34 family, including miR-34a, miR-34b, and miR-34c, which is robustly induced by DNA damage and oncogenic stress in

a p53-dependent manner (78–80). As a p53 downstream target, miR-34 causes apoptosis and cellular senescence, highlighting the biological consequences of p53-mediated microRNA expression. More recently, miR-605 has been shown to be a member of the p53 tumor suppressor network because it is transcriptionally activated by p53 (81). Of interest, miR-605 is able to suppress MDM2, thus forming a positive feedback loop. On the other hand, miR-17-92 cluster is a novel target for p53-mediated transcriptional repression under hypoxia (82). Similarly, let-7 is also repressed by p53 through interaction with a region upstream the let-7 gene (83). Finally, we and other groups have shown that p53 transcriptionally induces miR-145 which will be discussed in more detail in the next section. Together, these studies demonstrate p53 as an important regulator of microRNA expression.

miR-145

We take miR-145 as an example to illustrate the importance of microRNAs in tumorigenesis through the Akt pathway. We have shown that miR-145 is a direct target for p53 and it serves a critical modulator for p53-mediated Myc repression (12). Thus, miR-145 functions as an Akt downstream effector. Downregulation of miR-145 was reported in different types of tumors (84–87), suggesting its role in controlling cell proliferation. The tumor suppressive role of miR-145 in colon cancer was first reported by Shi *et al.*, (88, 89) who showed growth inhibitory role of miR-145 in two colon cancer cell lines in cell culture model. Specifically, miR-145 suppresses insulin receptor substrate-(IRS-1) through binding to its 3'-UTR. We used a xenograft mouse model, demonstrating the tumor suppressive role of miR-145 in colon cancer HCT-116 cells in vivo (12). Consistent with these findings, miR-145 can also significantly reduce cell growth in B-cell lymphoma cell lines (90). The underlying mechanism of tumor suppressive role of miR-145 may have to do with its ability to target multiple genes involved in cell proliferation. For example, several oncogenes have been shown to be direct targets of miR-145 and these include c-Myc, and its downstream genes such as cyclin D1 and *elF4E*, leading to cell cycle arrest (12). In breast cancer, ectopic expression of miR-145 inhibits cell growth in MCF-7 cells by induction of apoptosis; in contrast, this inhibitory effect is not obvious in non-tumorigenic MCF10A cells which express a high level of miR-145 (91). In consistent with these findings, miR-145 can also cause a significant growth inhibition in cancer cells expressing wild type p53 (92), which is associated with PARP cleavage and apoptotic death. Moreover, miR-145 can cause upregulation of p53-regulated genes such as PUMA and p21 (92). It is well known that induction of these genes can promote apoptosis or cell cycle arrest. In addition, miR-145 may also play a role in other types of cancers including lung cancer (93, 94), and urothelial carcinoma and colon carcinoma respectively (95, 96). Of interest, miR-145 induces cell death in both caspase-dependent and caspase-independent manner which is likely through targeting multiple oncogenesis related genes.

Regulation of miR-145—Available evidence indicates that miR-145 can be regulated at both transcriptional and posttranscriptional level. Our group was the first one who reported a transcriptional induction of miR-145 by p53 in response to stress such as serum starvation or anticancer drugs (12). For example, suppression of Akt activity by PI3K inhibitor Ly294002 substantially increases the p53 level and at the same time it induces miR-145 (12). Moreover, this p53-mediated induction of miR-145 depends on the ability of p53 bind to the miR-145 promoter. Although there are two putative p53 response elements in the upstream region of miR-145, only response element 2 is functional. This p53-mediated regulation of miR-145 was also confirmed by other groups (77, 92, 97) in breast, colon and cervical cancer cell lines. In addition to transcriptional regulation, Suzuki *et al.*, (77) demonstrated that p53 can also regulate miR-145 processing, providing a novel mechanism of posttranscriptional regulation of miR-145. In this regard, p53 interacts with Drosha processing complex through association with p68 and facilitates the processing of primary

transcript of miR-145. Interestingly, only wild type p53, but not mutant p53, associates with the complex containing p68 and miR-145. A clinical implication of this finding is the possibility that downregulation of miR-145 in tumors carrying null or mutant p53 might be in part due to the defect of miR-145 processing. In addition, the miR-145-mediated pro-apoptotic effect appears to be dependent on p53 activation which can, in turn, stimulate miR-145 expression (92). This may provide a clue as to why expression of miR-145 is low in tumors where p53 is also lost. However, this mechanism cannot explain why miR-145 is downregulated in tumors or cancer cell lines carrying wild type p53, implying that other mechanisms might also be involved. In support of this notion, we found that MCF10A cells express a much higher level of miR-145 than MCF-7 cells although both cell lines express wild type p53 (98). More recently, miR-145 as well as miR-143 was shown to target MDM2; both microRNAs can be posttranscriptionally activated by p53, thus forming a microRNA-MDM2-p53 feedback loop (99). Together, these studies demonstrate that miR-145 is regulated in a p53-dependent manner.

In addition to p53 and Foxo, several transcriptional factors, such as RREB1 (100), have been implicated in the regulation of miR-145. However, it is unclear as to why miR-145 is frequently downregulated in many types of tumors, including those carrying a mutant p53. Our recent study suggests that CCAAT/enhancer-binding protein-beta (C/EBP- β) plays a role in suppression of miR-145. In particular, while C/EBP- β is able to inhibit the ability of p53 to induce miR-145 in the wild type p53 background, it also suppresses miR-145 through the Akt pathway in the mutant p53 background (98). For example, we identify Akt as a potential upstream regulator of C/EBP- β , as supported by several lines of evidence. In particular, activation of Akt correlates with phosphorylation of C/EBP- β ; and resveratrol induces miR-145 at the concentration it decreases pAkt and phosphorylation of C/EBP- β . Therefore, these results highlight the importance of Akt in regulation of miR-145.

MicroRNAs as regulators of Akt activity

Although several factors upstream Akt have been shown to be targets for microRNAs, our main focus is on PTEN because PTEN is the most important negative regulator of Akt activity and a number of microRNAs are capable of targeting PTEN.

MicroRNAs capable of targeting PTEN

At the upstream of Akt, PTEN is the most important player not only because PTEN as a phosphatase functions to block the formation of PIP3 from PIP2, but also PTEN activity is frequently lost in many types of cancer, leading to increased cell survival and cell cycle progression. Although there are many factors that could account for this downregulation, evidently, one possibility is the upregulation of PTEN-targeting microRNAs. In this regard, miR-21 was the first microRNA identified to be able to directly target PTEN (101). miR-21 was shown to be upregulated in human hepatocellular cancer. Suppression of miR-21 by the miR-21 inhibitor increases PTEN and decreases tumor cell proliferation, migration, and invasion. Ectopic expression of miR-21 enhances cell migration in normal human hepatocytes (101). Targeting of PTEN by miR-21 is further demonstrated recently using the miR-21 knockout mouse model. Compared to wild type mice, the miR-21-knockout mice are more resistant to carcinogen-induced papilloma formation. In particular, several known miR-21 targets including PTEN are upregulated in miR-21-knockout keratinocytes (102).

In fact, miR-21 is not the only microRNA capable of directly targeting PTEN. miR-221/222 are known as oncogenic microRNAs and they are frequently overexpressed in a variety of tumors. Although a number of genes are targeted by these two microRNAs, a recent study has identified PTEN and TIMP3 as critical targets for aggressive non-small cell lung cancer and hepatocarcinoma cells in such a way that they activate the Akt pathway and

metallopeptidases (103). Similarly, miR-22 directly targets PTEN and at the same time, miR-22 itself is upregulated by Akt, suggesting that miR-22, PTEN and Akt form a feed-forward loop (104). This may explain why miR-22 activates Akt activity in response to growth factor stimulation.

Akt signaling is also involved in other cellular functions, one of which is muscle differentiation. Targeting PTEN by microRNAs may also impact these functions. For example, miR-486 is a muscle-enriched microRNA which is under control of myocardin-related transcription factor-A (MRTF-A) and serum response factor (SRF) (105). Of interest, miR-486 can target both PTEN and Foxo1. As a result, Akt signaling is significantly enhanced by suppression of miR-486. Hence, miR-486 is a potential modulator of Akt signaling (105). In addition, PTEN can be targeted by an intronic miR-106b~25 cluster which is imbedded in its host gene MCM7. Through targeting PTEN, they are able to promote prostatic intraepithelial neoplasia in transgenic mice (106).

MicroRNAs capable of targeting other negative regulators of Akt

In addition to PTEN, several phosphatases can also inactivate Akt. They include PH domain leucine-rich repeat protein phosphatase (PHLPP), which can be targeted by miR-190 so that it affects arsenic-induced carcinogenesis (107). The protein phosphatase 2A subunit B (PPP2R2A) can be targeted by miR-222 in hepatocellular carcinoma; suppression of miR-222 increases its level (108). Similarly, miR-200c has been implicated in resistance to chemotherapy among esophageal cancer because suppression of miR-200C is associated with increased expression of PPP2R1B and a reduced expression of pAkt (109).

miR-101

miR-101 has been previously shown to function as a tumor suppressor (110–112) because miR-101 is able to specifically target the histone H3K27 methyltransferase EZH2, a key gene required for polycomb repression complex 2. In particular, the genomic loss of miR-101 in cancer may cause upregulation of this gene and dysregulation of epigenetic pathways, leading to cancer progression (110). In addition, the histone H3 demethylase NDY1 can synergize with EZH2 to silence miR-101 where FGF-2 induces NDY1 via CREB phosphorylation and activation (113). Furthermore, miR-101 is able to target DNA repair genes such as DNA-PKcs and ATM. Ectopic expression of miR-101 sensitizes the tumor cells to radiation in vitro and in vivo (114).

Despite these findings, surprisingly, during the selection for microRNAs responsible for estrogen independent growth, we identified that miR-101 was among those microRNAs enriched through in vivo selection for microRNAs conferring estrogen independent growth (115). Basically, we generated a microRNA library carrying over 330 microRNA precursors which were cloned into a lentiviral vector. We then infected them as a mixed pool into MCF-7 cells. It is well known that MCF-7 cells express estrogen receptor (ER) and their growth is dependent on estrogen (116). For example, MCF-7 cells usually do not grow tumors in immune-compromised mice unless estrogen is provided. Therefore, this allows us to screen our microRNA library for those microRNAs that promote estrogen independent growth. Through this approach, we showed that miR-101 is capable of promoting estrogen independent growth and conferring tamoxifen resistance. Although several microRNAs have been shown to promote tamoxifen resistance by targeting estrogen receptor, including miR-206 (117, 118), miR-221/222 (119, 120) and miR-22 (121), miR-101 does not impact ER expression or ER activity, implying that dysregulation of miR-101 in tumors could lead to intrinsic resistance to tamoxifen regardless of ER status.

To determine the underlying mechanism by which miR-101 promotes estrogen independent growth, we interestingly discovered that miR-101 activates Akt, which is likely through targeting MAGI-2. MAGI-2 is a scaffold protein, a member of the membrane-associated guanylate kinases (MAGUK) family (122) involving the recruitment of PTEN to the membrane complex and to regulate PTEN activity (123). Once MAGI-2 is suppressed by miR-101, PTEN becomes phosphorylated. The phosphorylated PTEN is no longer able to function as an active phosphatase to inhibit conversion of PIP3 to PIP2. A consequence of that event is the activation of Akt (Fig. 2). Of great interest, estrogen deprivation increases the miR-101-mediated PTEN phosphorylation. This may explain why miR-101 inhibits cell growth in normal medium, however, it promotes cell growth in estrogen free medium (115).

As the major downstream target of PI3K-mediated signaling pathway in response to growth factors and cytokines, Akt is known to be influenced by many factors, including growth factors or their corresponding receptors, causing different biological consequences (124). A clinical implication of this finding is the possibility that certain breast tumor cells may develop an estrogen independent growth mechanism similar to what we described here because post-menopausal women often have an increased breast cancer risk, although their estrogen levels are usually decreased at that time. Tumor cells might have either totally lost or reduced their level of ER expression, which could account for an increase in tamoxifen resistance; however, dysregulation of microRNAs such as miR-101 might occur in the other tumor cells, which could cause estrogen independent signalling activation such that these tumor cells would be more aggressive and become hormone refractory. Therefore, it is of interest to determine whether miR-101 is dysregulated in these tumors and whether it contributes to the clinical tamoxifen resistance.

miR-145 and miR-101 are part of the microRNA-Akt regulatory network

Through the above discussions on miR-145 and miR-101, it becomes clear that while miR-145 serves as an Akt downstream effector, miR-101 regulates Akt activity in part by targeting MAGI-2. Consequences of the microRNA-mediated gene regulation include cell proliferation, tumorigenesis, estrogen independency (EI) and tamoxifen resistance (TR) (Fig. 3). For example, miR-101-mediated upregulation of Akt may facilitate the tumor cells to grow in the absence of estrogen, which otherwise would inhibit their growth. Regarding miR-145, since Akt controls mitogenic growth and proliferation, growth factors activate Akt; in contrast, stresses such as serum starvation or DNA damaging agents (e.g., Doxo) or PI3K inhibitors (e.g., LY294002) suppress its activity, leading to activation of p53. As a result, the activated p53 induces miR-145 and downstream events. Although p53 can repress c-Myc by a transcriptional mechanism (125, 126), our results suggest that miR-145 may play a more important role in this aspect because anti-miR-145 can rescue the vast majority of the p53-mediated repression (12). In addition to p53-mediated repression, c-Myc can enhance p53 expression through p19^{Arf} (127), which forms a feedback loop. In addition, our recent study further suggests that C/EBP- β is able to negatively regulate miR-145 (98). Therefore, this is a complex regulatory system that the cell may use to balance the “yin” and “yang” effect (128). Interruption of this balance, such as the downregulation of miR-145, could lead to cell malignancy.

Perspective

Give the importance of the PI3K/Akt pathway for normal cellular functions, alterations of this pathway could lead to a variety of human disorders including cancer. We also learn that microRNAs can play a fundamental role in regulation of diverse cellular pathways. In particular, a number of microRNAs are associated with the Akt pathway; data from our laboratory highlight the involvement of miR-145 and miR-101 in this regulatory network.

However, there are still many questions to be addressed. For example, little is known as to how Akt regulates miR-145 expression in tumor cells, leading to downregulation of miR-145 in various types of tumors. Although our recent studies suggest that C/EBP- β contributes to downregulation of miR-145 in tumors through Akt, the underlying mechanism is still not fully understood. On the other hand, miR-101 activates Akt in such a way that it may promote estrogen independency and tamoxifen resistance. However, it remains to be determined regarding the clinical relevance of miR-101-mediated Akt activation. Therefore, it would be of interest to determine how Akt and microRNAs interact and work together to exert their functions, and to identify important inter-players in different cellular contents. Hopefully, these issues will be addressed in the near future because this knowledge will aid in the identification of novel cancer biomarkers and therapeutic targets, and improve cancer diagnosis and treatment.

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Fig. 1.

As a key downstream target of PI3K-mediated signaling pathway in response to extracellular stimuli such as growth factors through receptor tyrosine kinase (RTK), Akt plays an important role in regulation of diverse cellular processes. In this pathway, several key factors, such as PTEN, Foxo and p53, are the potential targets for microRNA silencing. At the same time, as transcription factors, Foxo and p53 are able to regulate microRNA expression.

Fig. 2. miR-101 activates Akt by targeting MAGI-2, a scaffold protein associated with membrane, which is required for PTEN function. Of interest, estrogen deprivation enhances this effect because the level of pAkt is much higher in estrogen (E2) free medium than in E2 containing medium.

Fig. 3.
A simplified working model for Akt-associated microRNAs. miR-145 and miR-101 serve as examples. See text for detail explanation. EI, estrogen independency; TR, tamoxifen resistance.