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Review

Apoptosis and the target genes of microRNA-21

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Abstract

MicroRNA-21 (miR-21) is frequently up-regulated in cancer and the majority of its reported targets are tumor suppressors. Through functional suppression, miR-21 is implicated in practically every walk of oncogenic life: the promotion of cell proliferation, invasion and metastasis, genome instability and mutation, inflammation, replicative immortalization, abnormal metabolism, angiogenesis, and evading apoptosis, immune destruction, and growth suppressors. In particular, miR-21 is strongly involved in apoptosis. In this article, we reviewed the experimentally validated targets of miR-21 and found that two thirds are linked to intrinsic and/or extrinsic pathways of cellular apoptosis. This suggests that *miR-21* is an oncogene which plays a key role in resisting programmed cell death in cancer cells and that targeting apoptosis is a viable therapeutic option against cancers expressing miR-21.

Key words MicroRNA, microRNA-21, apoptosis, cancer

MicroRNAs (miRNAs) are small ribonucleic acid molecules 21 to 25 nucleotides in length. After transcription as primary miRNAs, they are processed into precursor miRNA (pre-miRNAs) by the enzyme Drosha, exported from the nucleus by exportin-5 and further processed into their mature form by Dicer. They are then incorporated into the RNA-induced silencing complex (RISC), which primarily binds the 3' untranslated region (3'UTR) of target mRNA in an miRNA-directed sequence-dependent manner, promoting mRNA degradation at a post transcriptional level and, in certain cases, inhibiting the initiation of translation. By down-regulating oncogenes or tumor suppressors, miRNAs may function in either tumor suppressive or oncogenic roles.

MicroRNA-21 (miR-21) is a specific miRNA that is up-regulated in nearly all epithelial cell–derived solid tumors including breast, pancreas, lung, gastric, prostate, colon, head and neck, and esophageal cancers [1]. It is also reported to be up-regulated in hematological malignancies such as leukemia [2], lymphoma [3], and multiple myeloma [4]. miR-21 is overexpressed in glioblastoma [5], osteosarcoma [6], and spermatocytic seminoma [7]. Thus, miR-21 appears to be the only miRNA or the only gene that is found to be overexpressed in all major classes of human cancers derived from epithelial cells, connective tissues, hematopoietic cells, germ cells, or nervous cells, supporting the premise that *miR-21* is a ubiquitous oncogene.

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Hallmarks of Cancer

The hallmarks of cancer, recently updated by Hanahan *et al.*[8], include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming energy metabolism, and evading immune destruction. In addition, genome instability and tumor-promoting inflammation are considered two enabling characteristics of cancer [8]. Scores of miR-21 target genes have been identified, and their functions in cancer have been revealed. Interestingly, these targets have been found to play a key role in virtually every one of the ten biological capabilities acquired or needed for tumor development (Figure 1). However, the vast majority of studies using cell lines, and two transgenic and knockout mouse models of *miR-21*, support the notion that miR-21 exerts its oncogenic function predominantly through the inhibition of cellular apoptosis [12,13]. Thus, we review the experimentally confirmed targets of miR-21, placing specific emphasis on their effects on apoptosis (Figure 2 and Table 1).

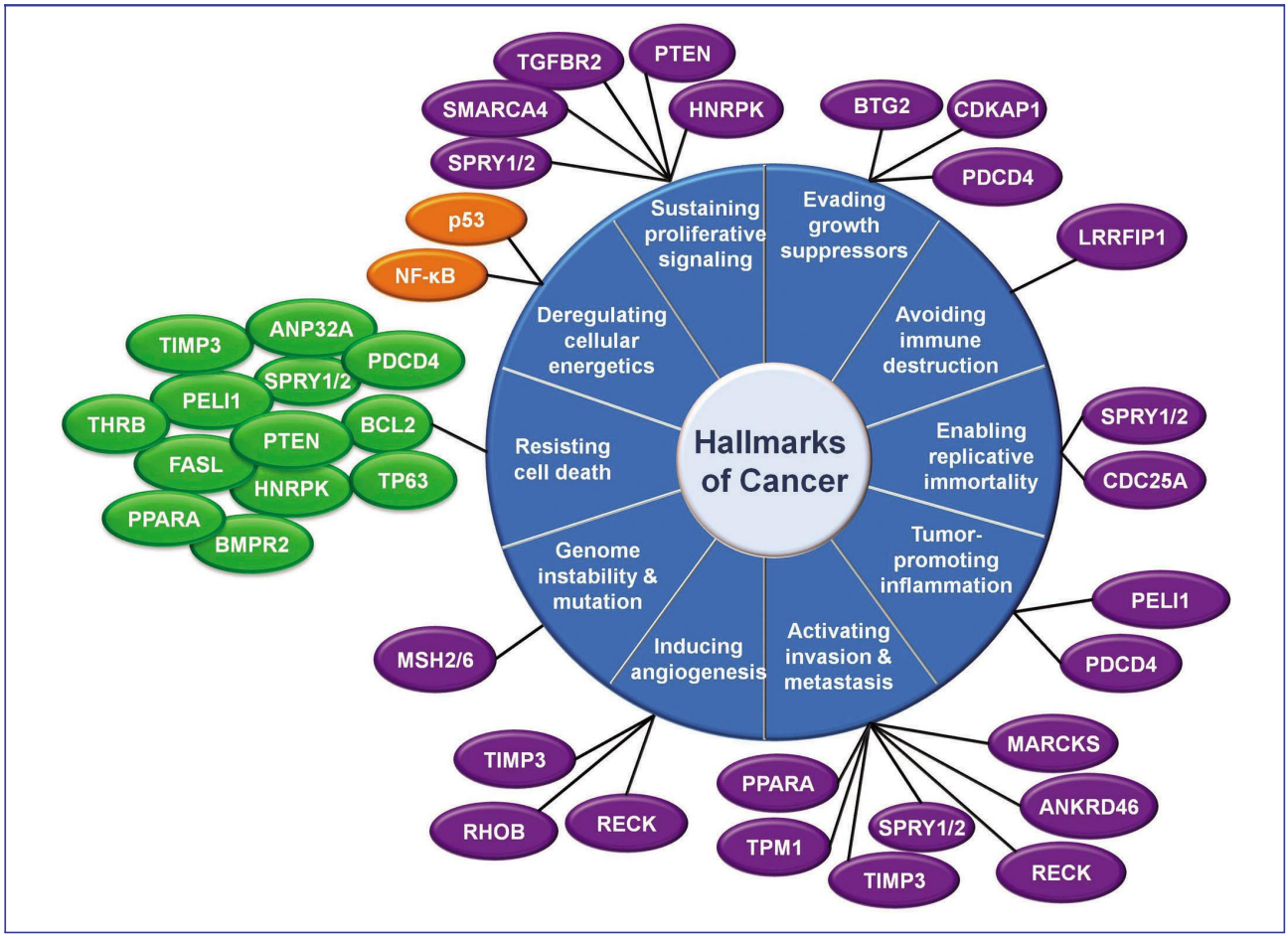

Figure 1. **MicroRNA-21 (miR-21) target genes and the next-generation hallmarks of cancer**. The eight hallmarks and two enabling characteristics (genome instability and inflammation) of cancer were described by Hanahan *et al*.[8]. Target genes shown in green are specific to apoptosis with other targets shown in purple. miR-21 is a negative regulator of p53 signaling[9], and NF-κB signaling is promoted by miR-21[10]. p53 inactivation and NF-κB activation are implicated in deregulation of glucose flux and oxidative phosphorylation[11]. It is notable that several targets contribute to more than one hallmark, and that virtually every hallmark involves at least one miR-21 target.

Apoptosis

Apoptosis is exerted through two pathways: an intrinsic or mitochondrial pathway and an extrinsic pathway induced by death ligands binding to cell surface receptors. Both pathways ultimately result in the activation of effector caspases, thiol proteases that cleave after aspartic acid residues. These apoptosis-specific proteases cleave structural proteins, signal transducers, regulators of transcription, repair factors, and many other targets within the cell. The apoptotic cell prepares itself for phagocytosis by actively flipping phospholipids, specifically phosphatidyl serine, from the inner to the outer leaflet of the cell membrane, creating a signal for phagocytosis by macrophages. As cells age or are damaged, the natural response is apoptosis. However, in many cancer cells, the apoptotic process is disrupted, allowing cells to survive and proliferate with damaged or mutated DNA. By analyzing the effects of miR-21 on this crucial regulator of cellular health, its role in tumorigenesis may be further elucidated. Beyond caspase-dependent apoptosis, there are other forms of cell death, such as the caspase-independent pathway of apoptosis, necrosis, and autophagy.

MiR-21 Targets

PDCD4

Programmed cell death 4 (Pdcd4) binds eIF4e to regulate translation, thus acting as a tumor suppressor. In breast cancer cells, miR-21 was up-regulated via stimulation of Her2/neu, a receptor tyrosine kinase [14]. Her2/neu-positive cells exhibit a down-regulation of

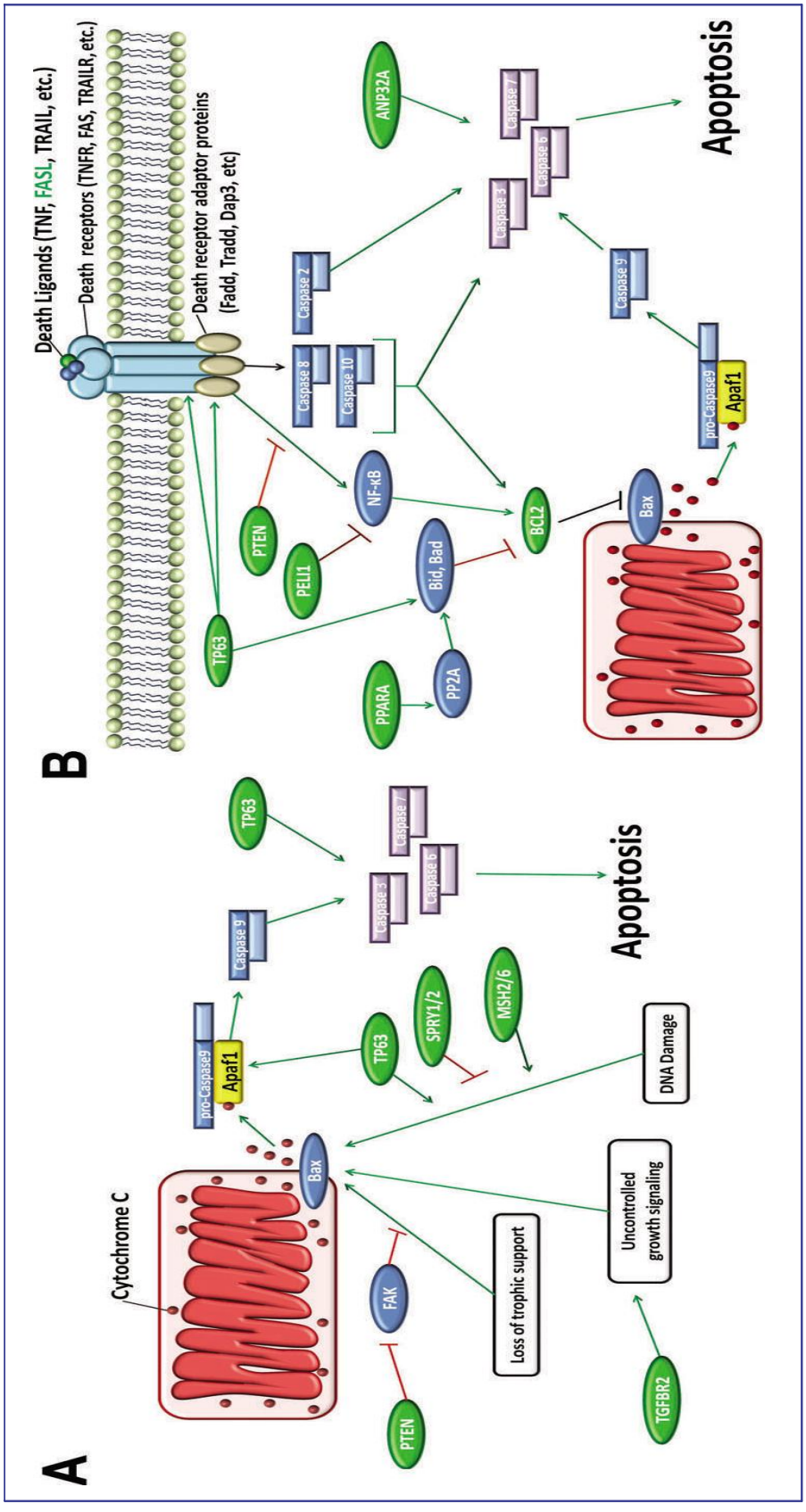

Figure 2. **Convergence of miR-21 target genes and the apoptosis pathway**. miR-21 targets are shown in green. A, the intrinsic apoptosis pathway. Signals within the cell induce Bax, a BH123 domain containing protein, to permeabilize the mitochondrial membrane, allowing the release of cytochrome C. Cytochrome C binds and activates Apaf1, which in turn activates the initiator caspase 9 by cleavage and formation of the apoptosome (not shown). This allows cleavage and activation of the effector caspases 3, 6, and 7, leading to apoptosis. TAp63 acts in a compensatory role in p53-deficient cells to activate pro-apoptotic genes in response to DNA damage. Fak is a scaffolding protein that activates the PI3K/Akt pathway to avoid apoptosis. Inhibition of this by Pten allows for anoikis. Spy1 expression is associated with the inhibition of DNA damage signal cascades that initiate apoptosis. hMsh2 is a member of the mismatch repair complex that can signal within the DNA damage-induced apoptosis pathway. High levels of TGFBR2 stimulate apoptosis, potentially through stimulation of the Mapk pathway. B, the extrinsic apoptosis pathway. Death ligands such as TNF or FasL bind their cell surface receptors, causing them to tri- or oligomerize, causing recruitment of the adaptor proteins Fadd or Tradd, leading to the formation of the death inducing signaling complex (DISC, not shown) comprised of the intiator caspases 8, 10, or 2. These can then activate the effector caspases 3, 6, and 7, causing apoptosis. These death ligands can also signal the mitochondrial apoptotic pathway by activating BH3 domain containing proteins such as Bid and Bad, which inhibit the anti-apoptotic protein Bcl2. Bcl2 then releases BH123 domain containing proteins such as Bax, allowing release of cytochrome C, formation of the apoptosome by Apaf1, and induction of the caspase cascade through the initiator caspase 9. TAp63 induces apoptosis by activating signaling via death receptors and mitochondria. Pten inhibits TNF activation of NF-κB, which in this context would ordinarily activate transcription of anti-apoptotic genes. Ligands specific to PPARα induce apoptosis through up-regulation of PP2A, which positively affects Bad levels. FasL is an apoptosis inhibitor that signals through Fas, a death receptor. Anp32A is a tumor suppressor that regulates apoptosis through activation of caspase activity. Pellino-1 is involved in activating NF-κB which prevents apoptosis during the proliferation phase of hepatocytes after hepatectomy. Pdcd4 is involved in TLR4 signaling and signals apoptosis through IL-6 and NF-κB.

Table 1. Published microRNA-21 targets validated with experimental data

Gene	Full name	Apoptotic role	References
PTEN	Phosphatase and tensin homolog	Direct	[30, 33]
PDCD4	Programmed cell death 4	Direct	[16]
TPM1	Tropomyosin 1 (alpha)	Unknown	[34]
SPRY1	Sprouty homolog 1, antagonist of FGF signaling (Drosophila)	Indirect	[25]
SPRY2	Sprouty homolog 2 (Drosophila)	Indirect	[26]
RECK	Reversion-inducing-cysteine-rich protein with kazal motifs	Unknown	[35, 36]
BCL2	B-cell CLL/lymphoma 2	Direct	[37, 38]
MARCKS	Myristoylated alanine-rich protein kinase C substrate	Unknown	[39]
HNRPK	Heterogeneous nuclear ribonucleoprotein K	Indirect	[9]
TP63	Tumor protein 63	Direct	[9]
IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	Direct	[42]
JAG1	Jagged 1	Unknown	[44, 45]
BTG2	B-cell translocation gene 2	Unknown	[46]
LRRFIP1	Leucine rich repeat (in FLII) interacting protein 1	Unknown	[47]
BMPR2	Bone morphogenetic protein receptor, type II (serine/threonine kinase)	Direct	[49]
TGFBR2	Transforming growth factor, beta receptor II (70/80kDa)	Indirect	[51]
CDC25A	Cell division cycle 25 homolog A (S. pombe)	Unknown	[52, 53]
PELI1	Pellino homolog 1 (Drosophila)	Direct	[54]
ANKRD46	Ankyrin repeat domain 46	Unknown	[55]
CDK2AP1	Cyclin-dependent kinase 2 associated protein 1	Unknown	[57]
MEF2C	Myocyte enhancer factor 2C	Unknown	[58]
MSH2	MutS homolog 2, colon cancer, nonpolyposis type 1 (*E. coli*)	Indirect	[59]
MSH6	MutS homolog 6 (*E. coli*)	Indirect	(59)
PPARA	Peroxisome proliferator activated receptor alpha	Direct	[61]
RASGRP1	RAS guanyl releasing protein 1	Unknown	[62]
FASLG	Fas ligand (TNF superfamily, member 6)	Direct	[63]
TIMP3	TIMP metallopeptidase inhibitor	Direct	[64, 65]
ANP32A	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	Direct	[66]
SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	Possibly direct	[66]
THRB	Thyroid hormone receptor, beta (erythroblastic leukemia viral (v-erb-a) oncogene homolog 2, avian)	Unknown	[68]

Pdcd4 [15]. In colorectal cells, the level of miR-21 and Pdcd4 expression were shown to have an inverse correlation. A luciferase construct showed down-regulation of the *PDCD4* promoter activity upon overexpression of miR-21, which is due to the direct targeting of the 3'UTR [16]. Pdcd4 inhibition by miR-21 causes an increased invasion in colorectal cells [17]. In addition, cervical carcinoma and glioblastoma cells have been used to demonstrate that miR-21 directly targets Pdcd4, thus causing tumorigenic properties [18,19]. A study by Talotta *et al.* [20] showed AP-1 is a transcription factor that induces miR-21 expression in response to Ras signaling. Through this pathway, Ras inhibits Pten and Pdcd4. Pdcd4 has also been shown to be a mediator of LPS-induced apoptosis [21]. Signaling of LPS through Toll-like receptor 4 (TLR4) causes an increase in the expression of Pdcd4. In response to LPS signaling, induction of IL-6 and NF-κB induces apoptosis and is dependent on Pdcd4 expression. In addition to inducing apoptosis, NF-κB also induces the expression of miR-21, forming a negative feedback loop by targeting Pdcd4. In this way, Pdcd4 responds to extrinsic signals to induce apoptosis. While LPS signaling differs from apoptotic signaling in tumorigenesis, Pdcd4 and miR-21 provide key links between inflammation and oncogenesis [22].

SPRY1 and SPRY2

Sprouty (SPRY) family members mediate receptor tyrosine kinase signaling in response to growth factors. As such, they modulate the map kinase (Mapk) pathway. Spry1 has been found to inhibit DNA damage response pathways, and to interact with and stimulate cell cycle progression factors such as Cdk1. Spry1 has also been found to prevent inhibition of Cdks by Cdk inhibitors such as p21. By avoiding DNA damage-induced apoptosis and the cell cycle checkpoint, Spry1 can function as an oncoprotein, and indeed its over-expression has been demonstrated to be correlated with breast cancer [23,24]. Spry1 also plays an important role in cardiac disease models. By inhibiting Spry1 in cardiac fibroblasts, miR-21 augments the Erk-Mapk pathway, affecting cardiac structure and function. By reducing miR-21 in a heart disease model, the Erk pathway was reduced, as

was interstitial fibrosis and cardiac dysfunction. In this way, miR-21 can contribute to myocardial disease through fibroblasts[25].

Spry2 is also inhibited by miR-21. Spry2 inhibits branching morphogenesis and neurite outgrowth in cardiocytes. Stimulation of the beta-adrenergic receptor induces miR-21 expression, targeting Spry2. This causes connections between cell-cell linker branches. Neurites enclose sarcomeres and connections between cardiocytes through gap junctions. Knockdown of miR-21 allows Spry2 to regulate these outgrowths and inhibits cell migration[26].

PTEN

Pten is a phosphatase and a tumor suppressor. It inhibits the Akt pathway by reversing the phosphorylation of phosphoinositide 3 kinase (PI3K) [27]. It has been investigated whether the loss of Pten results in oncogenesis in a tissue specific manner. While this remains controversial, there are certain functions of Pten that, thus far, appear to be differentially affected in different tissues. In breast cancer cells, Pten controls apoptosis and cell cycle arrest independently of each other [28]. Cells grown in low concentrations of growth factors were particularly susceptible to growth suppression and apoptosis by Pten. However, in glioblastoma cells, the presence of Pten mediated a switch from premature senescence to apoptosis resulting from ionizing radiation [29]. The control of apoptosis by Pten is often through the inhibition of Fak-induced PI3K signaling, specifically resulting in anoikis [30,31]. The apoptosis described in glioma exposed to ionizing radiation was linked to TNF signaling, either causing apoptosis through death receptor signaling or inhibiting apoptosis through activation of NF-κB, inducing the transcription of several anti-apoptotic proteins such as IAP1, IAP3, Bcl2, and Survivin. Pten inhibits the activation of NF-κB[32].

Pten is lost or mutated in many solid tumors. Meng *et al*. [30] used hepatocellular cancer cells to show that when miR-21 is inhibited, Pten expression increases and tumor cell proliferation, migration, and invasion decrease. Similarly, the transfection of non-cancerous cells with *miR-21* caused an increase in migration. miR-21 was shown to directly target Pten through sites within its 3'UTR. Pten is involved in regulating cell migration and invasion by regulating matrix metalloproteinase 2 (MMP2) and MMP9. The activity of both of these was affected by miR-21 concentration as well. Fak activity, which is involved in cell motility and survival, and is regulated by Pten, was also affected by miR-21[30]. Germline *PTEN* mutations are associated with heritable cancer. However, Pezzolessi *et al*. [33] found phenotypes of these cancers are affected largely by miR-21 regulation of Pten, regardless of the status of germline mutation. Overall, Pten can be directly linked to the extrinsic apoptotic pathway through mediation of TNFα signaling and the intrinsic apoptotic pathway by mediating expression of mitochondrial apoptosis factors.

TPM1

Tropomyosin (Tpm1) is important in muscle contraction, as it regulates actin mechanics and is regulated by troponin T. Zhu *et al*.[34] identified Tpm1 as a miR-21 target employing 2D gel electrophoresis. The overexpression of Tpm1 in the MCF-7 breast cancer cell line suppressed anchorage independent growth. As miR-21 is over-expressed in most breast cancers, the targeting of Tpm1 signifies its role in tumor progression by affecting cellular migration rather than apoptosis.

RECK

Reck is a membrane-anchored matrix metalloproteinase inhibitor. It has an important role in suppressing tumor cell invasion and metastasis by inhibiting MMPs from degrading basement membranes and allowing cancerous cells to enter the blood stream and invade other tissues. This action also inhibits the induction of angiogenesis. Thus, miR-21 increases MMPs to promote invasion. Reck has been implicated in gastric cancer cells and glioblastoma cells as a target of miR-21[35,36].

BCL2

Bcl2 is a direct participant in the apoptosis pathway, regulating caspase activity by helping to sequester cytochrome C in the mitochondria through inhibition of the mitochondria-permeabilzing protein Bax[37]. In contrast, another study indicates that miR-21 may down-regulate Bax and upregulate Bcl2, inhibiting apoptosis[38].

MARCKS

Myristoylated alanine-rich protein kinase c substrate (Marcks) is an actin filament crosslinking protein. Upon phosphorylation by PKC or binding to calmodulin, Marcks can be released from the plasma membrane and enter the cytoplasm. Marcks is involved in cell motility, mitogenesis and plasma membrane trafficking. Inhibition of Marcks, leading to dysregulation of actin structure, causes an increase in cell mobility and invasiveness. This phenotype was observed in response to direct targeting by miR-21 in prostate cancer cells[39].

HNRPK

Heterogeneous nuclear ribonucleoprotein K (Hnrpk) is a major pre-mRNA binding protein that regulates the

cell cycle and influences the processing, shuttling, transport, and metabolism of RNA. When miR-21 is inhibited in glioblastoma cells, an Hnrpk-dependent repression of growth and an increase in apoptosis and cell cycle arrest are observed[9]. Hnrpk is also involved in the regulation of transcription and translation, forming complexes with Tbp or Sp1 to enhance the transcription of c-Myc and c-Src, respectively. Through its abilities to both bind proteins and nucleic acids and be phosphorylated by several kinases, Hnrpk is also thought to act as a docking platform for relaying signals in the cell[40].

TP63

TAp63 is the full length, fully active splice variant of the *p63* gene (*TP63*). In the absence of the tumor suppressor p53, TAp63 compensates by responding to DNA damage and inducing apoptosis, and activating both death receptors and the mitochondrial apoptotic pathway [41]. The inhibition of TAp63 is associated with chemoresistance. When miR-21 is inhibited in glioblastoma cells, TAp63-dependent repression of growth, an increase in apoptosis and cell cycle arrest are observed[9,41].

IL12a

Interleukin-12p35 (IL-12a or IL-12p35) is the 35 kDa subunit of heterodimeric interleukin 12. This cytokine is responsible for inducing interferon gamma independently from T-cells. IL-12a also signals lymphocytes through Stat4. Mice deficient in IL-12a are susceptible to autoimmune problems. In addition, IL-12a is strongly implicated in airway inflammation. The overexpression of miR-21 in *IL-13* transgenic mice was found to be dependent on IL-13Rα 1. However, allergen-induced miR-21 independently mediated inflammation of IL-13Rα 1 and Stat6. In *IL-13* transgenic mice, IL-12a levels were decreased, found to be the result of miR-21 directly targeting the 3'UTR of *IL-12a* to down-regulate its transcription[42].

JAG1

Jag1 is the ligand for the Notch 1 cell surface receptor, which controls cellular fate. Upon ligand binding, a series of cleavages occur on the cytoplasmic side of the plasma membrane, resulting in the release of the Notch intracellular domain (Nicd). This domain then enters the nucleus where it interacts with the DNA binding protein CsI. This complex recruits transcription factors and releases co-repressors, allowing for transcription [43]. Notch 1 signaling is most closely associated with cell development and fate, such as the differentiation of progenitor cells into neurons or glia. As the complexity of the Notch 1 signaling pathway unfolds, some of its signaling processes become implicated in cancer. miR-21 regulates the differentiation of monocyte derived dendritic cells through targeting of Wnt1 and Jag1. Overexpression of either of these targets stalls differentiation and causes a decrease in endocytic capacity[44,45].

BTG2

B-cell translocation gene 2 (Btg2) is a cell cycle regulator and tumor suppressor. In laryngeal carcinoma cells, miR-21 levels are high, whereas Btg2 levels are low. These cells demonstrate elevated growth. However, the knockdown of miR-21 inhibits proliferation due to a loss of G_1-S phase transition, rather than an increase in apoptosis[46].

LRRFIP1

Leucine rich repeat (in FLII) interacting protein 1 (Lrrfip1) regulates TLR signaling and mediates the production of type 1 interferon via a β-catenin–dependent signaling pathway. In glioblastoma cells, miR-21 levels are elevated to repress Lrrfip1, contributing to tumor cell resistance to chemotherapy, specifically VM-26[47].

BMPR2

The bone morphogenetic protein receptor type 2 (BMPR2), a serine/threonine kinase, is part of the TGF-β superfamily, the ligands of which are bone morphogenetic protein (BMPs). BMPR2 is involved in endochondral bone formation and embryogenesis. Intriguingly, mutations are commonly associated with primary pulmonary venoocclusive disease and are observed in the majority of patients with familial idiopathic pulmonary arterial hypertension (IPAH). IPAH is characterized by proliferation of vascular cells. In both osteoblasts and pulmonary cells, BMP signaling triggers apoptosis through the activation of caspase 9 through Smad signaling. A decrease in Bcl2 protein levels is also observed, implicating the involvement of the mitochondrial apoptotic pathway. However, whether this repression is due to Smad signaling or changes in mRNA stability is not fully known. A study by Lagna *et al.* [48] has shown that BMPR2 mutations, which are commonly found in IPAH, inhibit the pro-apoptotic signaling effects of BMPR2 that normally occur in response to the binding of two of its ligands, BMP4 or BMP7. These mutations prevent the activation of caspases-8, -9, and -3 and result in the derepression of Bcl2. In human prostate carcinoma cells, Qin *et al.* [49] recently showed that through direct targeting of four predicted sites within BMPR2 mRNA, miR-21 down-regulates levels of BMPR2.

TGFBR2

TGFBR2 is a receptor for TGF-β. TGF-β receptors phosphorylate proteins, facilitating their translocation to the nucleus with the subsequent regulation of genes involved in proliferation. TGF-β signaling is mediated through a Smad signaling cascade or through a non-Smad Mapk pathway. It has recently been shown that TGFBR2 is responsible for stimulating the Mapk-Erk pathway, which may mediate apoptosis. More specifically, high levels of TGFBR2 mediate this pro-apoptotic response to TGF-β signaling [50]. Notably, many cancers that do not demonstrate mutations in any TGF-β signaling cascade members show down-regulated levels of TFGBR2, demonstrating the oncogenic potential of TGF-β. TGFBR2 is also an important contributor to adipogenic differentiation. Kim *et al.*[51] showed that after differentiation, miR-21 transiently increased, peaking at 3 days and returning to baseline after 8 days. During this period, TGFBR2 levels and Smad3 phosphorylation were reduced.

CDC25A

Cell division cycle 25A (Cdc25A) is a cell cycle regulator. miR-21 directly binds to the target site in the 3'UTR of Cdc25A and suppresses its expression, resulting in a modulation of cell cycle progression following stress, without affecting apoptosis [52]. In colon cancer cells, serum starvation and DNA damage induces miR-21, which is under-expressed in Cdc25A-overexpressing colon cancers. Consistent with this, hypoxia causes a decrease in Cdc25A protein levels in colon cancer cells. S-phase arrest is observed with a decreased mitotic population. Protein and mRNA levels of Cdc25A, specifically, are reduced. This decrease is dependent on p21 and miR-21, both of which are up-regulated in colon cancer cells during hypoxia[53].

PELI1

Pellino-1 is a ubiquitin ligase that forms a complex with IRAK1, IRAK4 and TRAF6 in order to stimulate NF-κB activity. NF-κB activity, induced through TLR, helps to prevent apoptosis in hepatocytes during proliferation following hepatectomy. However, NF-κB activity is not essential due to simultaneous proliferative signaling pathway activation. Marquez *et al.*[54] found that inhibition of Pellino-1 by miR-21 causes the abrogation of NF-κB activity and that miR-21 is up-regulated in hepatocytes during proliferation in response to NF-κB. In this context, miR-21 is working in a feedback loop by targeting Pellino-1 to regulate apoptosis via NF-κB during liver regeneration.

ANKRD46

Ankyrins are adaptor proteins that attach membrane proteins to the cytoskeleton. Cytoskeletal proteins are important to the oncogenic process of migration and in disease states such as muscular dystrophy. Yan *et al.*[55] recently demonstrated that in breast cancer cells, miR-21 is up-regulated, corresponding with a down-regulation of Ankrd46, which leads to an increase in proliferation and migration.

CDK2AP1

miR-21 down-regulates the tumor suppressor gene *CDK2AP1*, which is known as p12, and stimulates cell proliferation and invasion. In the human immortalized keratinocyte cell line, HaCaT, p12 was found to be negatively regulated by miR-21. Inhibition of miR-21 caused a decrease in proliferation and invasion. p12 is a growth suppressor in keratinocytes. TGF-β1 induces the expression of p12, which binds DNA polymerase-α and Cdk2 to inhibit their activity. The inhibition of this protein has also been shown to cause increased Rb phosphorylation[56,57].

MEF2C

Monocyte enhancer factor 2C (Mef2c) is an important transcription factor in neuron function. Dementia is frequently caused by human immuno-deficiency virus (HIV), and miR-21 is often overexpressed in HIV patients. In this context, miR-21 can lead to pathological defects. miR-21 targets the mRNA of Mef2c in neurons, and Mef2c has been shown to be significantly down-regulated in the neurons of HIV dementia patients[58].

MSH2 and MSH6

miR-21 directly targets human mutS homologue 2 and 6 (hMsh2 and hMsh6), which together form the core mismatch recognition repair complex (MMR). This dysfunction contributes to colorectal cancer cell resistance to 5-fluoruracil, a chemotherapy drug that causes DNA damage, stimulating a repair/apoptosis pathway. By avoiding this pathway, damaged cells are allowed to continue proliferating. The down-regulation of this MMR complex may serve as an indicator of therapeutic efficacy in colorectal cancer[59].

PPARA

Peroxisome proliferator-activated receptor alpha (PPARα) is a nuclear receptor that acts as a transcription factor to regulate the expression of genes

involved in cellular differentiation, development, metabolism and tumorigenesis. Ligands specific to PPARα induce apoptosis by increasing the levels of the pro-apoptotic factor, Bad [60]. miR-21 plays a significant role in hypoxia-induced cell migration. In hypoxia, cells exhibit very low levels of PPARα due to direct targeting and suppression by miR-21. This suppression leads to hypoxia-induced proliferation and migration[61].

RASGRP1

RAS guanyl-releasing protein 1 (Rasgrp1) is a diacylglycerol (DAG)-regulated guanine exchange factor for Ras. miR-21 directly targets Rasgrp1, which is upstream of the Erk/Mek pathway that signals DNA methyltransferase activity. This was found to be important in systemic lupus erythematosus, an autoimmune disease caused by genetic alterations such as abnormal DNA methylation[62].

FASLG

Fas ligand (FasL) is a transmembrane protein that is a member of the TNF family and induces apoptosis by binding its receptor. When the TNFR binds its ligand, it may either induce apoptosis through the death-inducing signaling complex (DISC) or inhibit apoptosis by activating NF-κB to up-regulate transcription of anti-apoptotic factors such as Bcl2 (see Pten). During hypoxia, miR-21 is down-regulated and FasL is up-regulated due to lack of targeting of the FasL by miR-21. During hypertrophy and cancer growth, miR-21 is up-regulated, leading to a decrease in FasL and its pro-apoptotic signaling. Akt signaling reverses the hypoxia-induced repression of miR-21, which inhibits FasL[63].

TIMP3

Tissue inhibitor of metalloproteases 3 (Timp3) is a metalloprotease inhibitor that inhibits extracellular matrix degradation. Its levels are inversely correlated with miR-21 in breast cancer, possibly due to direct targeting by miR-21. The down-regulation of miR-21 can lead to apoptosis caused by increased amounts of caspases 9 and 3. This caspase induction may be a result of Timp3 activation[64,65].

ANP32A

Acidic nuclear phosphoprotein 32 family member A (Anp32A) is a protein that exerts its anti-apoptotic effect by inhibiting protein phosphatase 2A (PP2A) and histone acetyl transferase activity, as well as activating caspase activity to stimulate apoptosis. In the human prostate carcinoma cell line LNCap, Anp32 levels were notably decreased in response to direct targeting of miR-21. The knockdown of Anp32A resulted in an increased invasiveness that resembled the effects of overexpression of miR-21. Similarly, a glioblasoma cell line, A172, showed direct targeting of Anp32A by miR-21, causing escape from apoptosis[66].

SMARCA4

Swi/Snf related, matrix associated, actin dependent regulator of chromatin A4 (Smarca4) is also known as Brg1. It is known to interact with the chromatin remodeling Swi/Snf complex and to bind Brca1. Schramedei *et al*. [66] found that along with Anp32A, miR-21 directly targets Smarca4 in LNCap and A172 cell lines. However, the oncogenic potential of Smarca4 is controversial. Schramedei *et al*. [66] have reported Smarca4's tumor suppressive properties, but Watanabe *et al*.[67] demonstrated Brg1 (Smarca4) as a regulator of Pten's tumor suppressive activities. In colorectal carcinoma cells, Brg1 was found to activate the Akt/PI3K pathway and induce Cyclin D activity, although the role of miR-21 in this context was not analyzed[67].

THRB

Thyroid receptors (TR) are ligand-dependent transcription factors that regulate cell proliferation, differentiation, and apoptosis. In mouse models, a truncated thyroid hormone receptor β (*THRB*) gene leads to thyroid cancer. Jazdzewski *et al*.[68] analyzed papillary thyroid carcinoma tissues and revealed a down-regulation of Thrb in 11 of 13 pairs and an up-regulation of miR-21 in almost all pairs. They further validated a bona fide miR-21 binding site in the 3'UTR of THRB, although cellular apoptosis was not evaluated in this work[68].

Concluding Remarks

miR-21 suppresses the expression of a large number of genes that participate directly or indirectly in the extrinsic or intrinsic apoptosis pathways to promote tumorigenesis. The omnipresent overexpression of miR-21, particularly in a mouse model, underscores the importance of targeting apoptosis as a therapeutic tool for cancer[12]. In sharp contrast to other critical oncogenes such as *MYC* and *KRAS* that are essential for embryo viability, the loss of miR-21 has no apparent phenotypes in mice [13,69]. In addition, the global overexpression of miR-21 does not induce tumors, whereas tissue-specific overexpression does [12], furthering debate over whether miR-21 up-regulation drives or accompanies tumorigenesis [70]. Nonetheless, investigation of the fundamental pathophysiology of miR-21 will continue, and exploring the therapeutic efficacy of miR-21 and its

target genes will persist.

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