Review

Crosstalk between microRNA30a/b/c/d/e-5p and the Canonical Wnt Pathway: Implications for Multiple Myeloma therapy

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Abstract

Dysregulation of transcription via the Wnt/β-catenin signaling pathway underlies the pathogenesis of a wide variety of frequent human cancers. These include epithelial carcinomas such as colorectal cancer and hematologic malignancies such as multiple myeloma. Thus, the Wnt/β-catenin pathway potentially offers an attractive target for cancer therapy. This approach, however, has thus far proved challenging because the pathway plays a number of critical roles in physiologic homeostasis and because drugs that broadly target the pathway have unacceptable side effects. miRNAs function as regulators of gene expression and have also been implicated in the pathogenesis of multiple myeloma and other human cancers, offering the promise of novel therapeutic approaches if they can be applied effectively in vivo. Because BCL9 is a critical transcriptional coactivator of β-catenin that is aberrantly expressed in many human cancers but is of low abundance in normal tissues, the Wnt/β-catenin/BCL9 complex has emerged as a promising and most likely relatively safe therapeutic target in cancers with dysregulated Wnt/β-catenin activity. This review discusses recent advances in the biology of Wnt inhibitors and the appealing possibility of a functional link between BCL9 and miRNA30a/b/c/d/e-5p that could be exploited for multiple myeloma therapy. Cancer Res; 74(19); 1–8. ©2014 AACR.

Introduction

Multiple myeloma is a cancer of terminally differentiated, malignant postgerminal center B cells. Multiple myeloma is characterized by clonal proliferation of long-lived plasma cells in the bone marrow, along with serum monoclonal gammapathy, and skeletal bone destruction partially because of inhibition of the Wnt/β-catenin signaling pathway in osteoblasts (1). It is preceded by a progressive premalignant condition termed Monoclonal Gammapathy of Undetermined Significance (MGUS; ref. 2). Despite recent advances in its treatment, multiple myeloma remains incurable, highlighting the need for sustained efforts to develop novel rationally designed therapeutics.

Significant effort has been devoted recently to the identification of molecular genetics events leading to this malignancy, with the twin goals of improving early detection and identifying new therapeutic targets. Unlike most hematologic malignancies, and more in common with solid neoplasms, multiple myeloma genomes are typified by numerous qualitative and quantitative chromosomal aberrations. Reflecting the increasing genomic instability that characterizes disease progression, metaphase chromosomal abnormalities are detected in only one third of newly diagnosed patients but are evident in the majority of those with end-stage disease (3). Extensive molecular (4), cytogenetic (5), and comparative genomic hybridization (CGH) analyses (6) have uncovered a number of recurrent genetic alterations, some of which have been linked to disease pathogenesis as well as clinical presentation and progression. The high-resolution views afforded by current genome-scanning platforms, such as array-CGH, SNP array, and whole-genome sequencing has led to the discovery of novel tumor suppressor genes and oncogene candidates involved in multiple myeloma pathogenesis (1, 7–10). Taken together, these efforts have uncovered a remarkably high degree of molecular heterogeneity among multiple myeloma tumors and have made us powerfully aware of the difficulties that will likely be faced in identifying molecular events consistently driving disease initiation and progression, and in designing effective targeted, and ultimately perhaps even personalized, therapies that will spare patients from side effects while at the same time simplifying patient selection tactics.

Role of the Canonical Wnt/β-Catenin Signaling Pathway in Multiple Myeloma Pathogenesis

The canonical Wnt/β-catenin pathway is a receptor-mediated signal transduction network required for normal embryonic development and adult tissue homeostasis. Its activity hinges on the expression, localization, and activity of β-catenin (11, 12). In the absence of Wnt ligands, β-catenin binds to
adenomatous polyposis coli (APC) protein, glycogen synthase kinase 3β (GSK3β), and axin to form a "destruction complex" that phosphorylates β-catenin, targeting it for proteosomal degradation. Binding of Wnt ligands to the lipoprotein receptors LRP5 and LRP6 inhibits the activity of the APC/GSK3β/axin complex, enabling nonphosphorylated β-catenin to undergo nuclear translocation and thereafter regulate transcription (13). Nuclear β-catenin associates with the lymphoid enhancer factor/T-cell factor (LEF/TCF) family of transcription factors to induce expression of genes involved in cell proliferation and survival, as well as migration and angiogenesis (11, 14).

The molecular genetics underlying Wnt/β-catenin activation in cancer are driven by mutations that enable β-catenin to escape the destruction complex and accumulate in the nucleus. These include loss of function mutations in the tumor suppressors APC and Axin as well as activating mutations in β-catenin itself (15, 16). Genetic assays in Drosophila as well as mammalian systems have demonstrated that the transcriptional activity of β-catenin largely depends on 2 recently discovered components, BCL9 and pygopus (PYG; refs. 17 and 18). Moreover, biochemical analysis has shown that nuclear β-catenin assembles in a quaternary complex, consisting of TCF, β-catenin, BCL9, and PYG, in which BCL9 binds directly to β-catenin and plays a role in targeting and retaining β-catenin in the nucleus, increasing its net nuclear concentration and, hence, its activity (19–23).

Remarkably, the canonical Wnt pathway is constitutively active in multiple myeloma and promotes tumor cell proliferation, disease progression, and resistance to chemotherapy (24–27); however, mutations in APC, axin or β-catenin have not been reported (8, 9). The absence of loss of function mutations in the tumor suppressors APC and Axin, as well as activating mutations in β-catenin itself, suggest that multiple myeloma may have alternate pathways for β-catenin activation (8, 9). Multiple myeloma cell lines have been shown to respond to the Wnt ligand Wnt3A, the GSK3β-inhibitor lithium chloride, and an active mutant form of β-catenin to with significantly increased proliferation and higher levels of nonphosphorylated nuclear β-catenin. Furthermore, growth of multiple myeloma cell lines can be blocked upon transfection with a dominant negative form of TCF4 (24, 26). In addition, Wnts induce migration and invasion of multiple myeloma plasma cells. These findings demonstrate that Wnt signaling is active in multiple myeloma, acts through β-catenin/TCF-regulated transcription, and responds to Wnt stimulants and/or inhibitors. Interestingly, using gene expression profiling analysis as well as in vitro and in vivo functional studies, we demonstrated that BCL9 is a bona-fide oncogene that is aberrantly expressed in human multiple myeloma as well as colon carcinoma but it not expressed in their normal cellular counterpart where they originate (14). We have shown that BCL9 enhances β-catenin-mediated transcriptional activity regardless of the mutational status of the Wnt signaling components, increases cell proliferation, migration, invasion, and the metastatic potential of tumor cells. Most importantly, BCL9 knockdown significantly increased the survival of mouse models of cancer by reducing tumor load, metastasis, and host angiogenesis through downregulation of e-Myc, cyclin D1, CD44, and VEGF expression by tumor cells. Together these findings suggest that deregulation of BCL9 is an important contributing factor to tumor progression. The pleiotropic roles of BCL9 and its restricted expression to multiple myeloma cells but not in normal plasma cells underscore its value as a drug target for therapeutic intervention in multiple myeloma and other malignancies associated with aberrant Wnt signaling (14).

The mechanism of pathologic Wnt signaling in multiple myeloma has been linked to posttranscriptional regulation of β-catenin and/or increased levels of BCL9, implicating this β-catenin cofactor as a bona-fide oncogene (14). Inhibition of the canonical Wnt pathway has been regarded as an attractive therapeutic approach for multiple myeloma (11). This approach, however, has raised some concerns because Dickkopf-1 (Dkk1), which specifically inhibits canonical Wnt signals by binding to the LRP6 component of the receptor complex on target cells, is actively secreted by multiple myeloma cells (28) and blocks differentiation of osteoblasts from mesenchymal precursor cells (29). This blockade results in a decrease in bone formation that is inadequate to match enhanced bone resorption in patients with multiple myeloma bone lytic lesions (28). Thus, despite the therapeutic benefits of blocking multiple myeloma cell proliferation, there was concern that systemic delivery of WNT inhibitors may be synergistic with Dkk1 and thereby worsen bone destruction in patients with multiple myeloma by inhibiting osteoblast differentiation.

Inhibitors of Wnt Transcriptional Activity in Cancer Therapy

Wnt/β-catenin activity underlies the pathogenesis of a wide range of common human cancers (30) including multiple myeloma (31), and has emerged as a promising target for cancer therapy. Several drugs that inhibit this pathway have been identified; however, none of them has successfully made it into clinical practice because they lack sufficient preclinical efficacy or are associated with dose-limiting toxicities. The latter are due, in part, to the fact that Wnt/β-catenin signaling plays critical roles in normal tissue homeostasis, narrowing the therapeutic window for broad pharmacologic targeting of the pathway.

Because β-catenin interacts with most of its protein partners via the same binding surface, identifying agents that can selectively disrupt its cancer-promoting activities while leaving its homeostatic functions (e.g., self-renewal and multipotency of hematopoietic and intestinal stem cells) intact poses a challenge (30). β-Catenin interacts with its transcription activators BCL9 and BCL9.2 via the same N-terminal domain, interacts with TCF/LEF via a central region that spans 12 armadillo repeats, and interacts with positive effectors, such as CBP, P300, TRRAP, and parafibromin or with negative ones, such as chibby, ICAT, and APC through its C-terminal domain (CTD; ref. 12). Thus, it has been difficult to identify agents that can selectively disrupt the interaction between a transcription
activator and β-catenin while leaving the CTD and core region undisturbed in order to preserve the WNT activity needed for normal homeostasis.

Small-molecule and peptide inhibitors of β-catenin/LEF/TCF interaction have been reported (11, 32, 33). Although these inhibitors block Wnt-specific transcriptional activity and reduce the growth of colorectal and multiple myeloma cells, they also induce severe bone marrow hypoplasia, anemia, and intestinal atrophy in treated mice, probably via disruption of homeostatic Wnt signaling in normal hematopoietic and intestinal stem cells (25). These therapeutic limitations may also reflect disruption of β-catenin–TCF and β-catenin–E-cadherin interactions, which are known to affect epithelial tissue integrity (34).

Other small molecules indirectly affect the Wnt pathway by interacting with other proteins that modulate effectors related to Wnt signaling, such as CBP (35), Porcupine (36), tankyrase (37), or casein kinase 1 (38), but are also associated with off-target effects and toxicity (39). Tankyrase inhibitors, which destabilize β-catenin by stabilizing Axin, have not been associated with toxicity. However, recent studies have shown that during Wnt stimulation, β-catenin become unresponsive to tankirase inhibitors. During sustained Wnt stimulation, LEF1 and B9L proteins accumulate inside the cell, protecting β-catenin from Axin-induced inactivation (39).

As an alternative strategy, selective direct targeting of β-catenin by disruption of BCL9/B9L/β-catenin complexes is strategically attractive because: BCL9 drives pathologic β-catenin transcriptional activity; the β-catenin binding site in BCL9 (or B9L) is unique and corresponds to the homology domain 2 (HD2). BCL9 is overexpressed at both mRNA and protein levels in tumor cells compared with cells of same histologic type derived from normal tissues (11, 14); elimination of BCL9/B9L–β-catenin interactions using stapled peptides (11) or inactivation of BCL9 and B9L genes by knockout approaches in the murine gut (40) and muscle (41) causes no overt phenotypic consequences, indicating that blockade of BCL9 function may not harm normal cells. Notably, BCL9-shRNA–mediated inhibition of β-catenin transcriptional activity decreased tumor cell proliferation, migration, and invasion by suppressing the expression of Wnt target genes (14). BCL9-targeted treatment increased the survival of mice with Xenograft multiple myeloma tumors by reducing both tumor load and metastasis (14); and a stable, conformationally rigid “stapled” peptide derived from BCL9-HD2 and carnosic acid have shown in vitro and in vivo antitumor activity (11, 42, 43). Although such stapled peptides do not exhibit pharmacokinetic profiles appropriate for further preclinical development, small molecules that similarly disrupt BCL9/B9L–β-catenin interactions may be therapeutically effective while causing minimal side effects. Of note in this regard, is that the small-molecule drug carnosate, which inhibits the action β-catenin by blocking its binding to BCL9, has not been associated with toxicity, and has yielded promising results in a preclinical mouse model of cancer with a dysregulated Wnt pathway (39).

Collectively, these data indicate that targeting the BCL9 component of aberrantly activated Wnt signaling in cancer may attenuate invasion, metastasis, and refractoriness to treatment, highlighting the importance of this pathway, and specifically of BCL9, as a platform for targeted drug discovery.

Role of miRNAs in Multiple Myeloma Pathogenesis

miRNAs are evolutionarily conserved, small noncoding RNAs that play key regulatory roles in mRNA translation, and also in mRNA degradation, by base-pairing, predominantly in the 3′-untranslated region (3′-UTR), to partially complementary sites of the mRNA (44, 45). Each miRNA can directly or indirectly target hundreds of transcripts (46, 47), and more than one miRNA can converge upon a single transcript target. Rapidly accumulating evidence indicates that miRNAs are involved in the initiation and progression of cancer (48–51). miRNAs act as key regulators of normal biologic processes such as development, differentiation, apoptosis, and cell proliferation, all of which have counterparts in cancer (52), and several of them are known to function as tumor suppressor genes or oncogenes (51, 53). These findings collectively support the notion that systematic investigation of miRNA function in cancer is needed and may yield novel targets for therapy.

Several independent studies have documented dysregulated expression of miRNAs in multiple myeloma. However, these studies have generated a certain degree of confusion in the field, because various studies have reported different miRNA families as being important in multiple myeloma, which may be due in part to the use of different experimental platforms, to different statistical methodologies to quantify miRNAs levels, or to the heterogeneity of the patients population and the sample size (54). In this review we are emphasizing especially these studies that show more consistency among the miRNAs and have been validated via molecular studies and actual therapeutic experiments (Table 1). A miRNA signature was firstly described in multiple myeloma that is distinct from MGUS and normal plasma cells, suggesting that altered expression of miR21-5p, miR106b-5p-25-3p, miR181a-5p/b-5p, and miR17-5p-92a-3p may be involved in multiple myeloma progression (51, 55). Two target genes of overexpressed miRNAs, SOCS-1 and p300-CBP, were identified as having a role in multiple myeloma pathogenesis (51). Although the molecular events involved in regulating expression of miRNAs are not entirely known, recent studies do point to the existence of such regulatory mechanisms, including interaction with the tumor microenvironment and DNA copy number alterations of chromosomal regions containing miRNAs, among others (56, 57). miR21-5p was found to be upregulated by IL6, which is secreted by the bone marrow microenvironment (58). Surprisingly, although monoallelic deletion of chromosome 13 is present in about 50% of patients with multiple myeloma (59), the miR17-5p-92a-3p cluster that resides on chromosome 13q31.3 was found to be highly expressed in patients with multiple myeloma, and in vivo studies suggested an oncogenic role for this miRNA cluster (51, 60). This suggests the possibility of alternative mechanisms of regulation for miR17-5p-92a-3p cluster expression. The oncogene c-MYC protein that is overexpressed in almost half of multiple myeloma cases (60), has been shown to directly upregulate expression of the
miR17-5p-92a-3p cluster (61), and silencing of c-MYC in multiple myeloma resulted in downregulation of miR17-5p-92a-3p and inhibition of cell growth (51), further supporting an oncogenic role for this miRNA cluster. Downregulation of miR15a-5p-16p residing in the minimal common region of deletion on chromosome 13q14 in multiple myeloma has also been associated with enhanced multiple myeloma cell proliferation (53). However, in other studies, correlation between decreased miR15a-5p-16p levels and 13q14 deletion in patients with multiple myeloma was not established (55, 62, 63). Even though there seems to be a discrepancy between them, these results suggest the possibility of multiple levels of regulation of miR15a-5p-16p expression, such as DNA copy number alteration of the chromosomal region where these miRNAs reside, transcriptional or posttranscriptional level, and other unknown mechanisms.

An association between distinct miRNA expression patterns and signaling pathways, such as p53, IGF, VEGF, NF-κB, and others, has also been described in multiple myeloma (64). A combination of mRNA and miRNA expression profiling has identified a miR–mRNA regulatory network with a distinct expression signature associated with high-risk multiple myeloma (65). Downregulation of miR192-5p, miR194-5p, and miR215-5p expression, in a subset of patients with multiple myeloma, has been correlated with transcriptional activation of p53 and modulation of MDM2 expression, suggesting that these miRNAs function as positive regulators of p53 with an important role in multiple myeloma development (66).

A link between miRNA and the Wnt pathway was first established by a genetic screen in Drosophila. miR8 was identified as an inhibitor of Wg signaling that directly targets wntless, a gene required for Wg secretion, repressing TCF protein levels as well as those of another positive regulator of the pathway, namely CG32767 (67). In a mammalian system, it was shown that miR135a/b-5p target the 3’-untranslated region of APC, suppressing its expression, and inducing downstream Wnt pathway activity. In addition, a novel mechanism for APC regulation other than mutations was described, involving the generation of premature stop codons that produced truncated APC proteins lacking β-catenin binding sites (68). In addition, it has been shown that the Wnt pathway can regulate miRNA expression. For instance, Wnt/β-catenin signaling regulates miR15a-5p/16p maturation but not its transcription. Overexpression of β-catenin inhibits the expression of mature miR15a and miR16 isoforms in early Xenopus embryos (69). The mechanism of Wnt control of miR15a and miR16 maturation is unknown, but perhaps works through a protein complex controlled by, or containing, β-catenin. Very recently, it was documented that members of the miR30-5p family, including miR30a-5p, miR30b-5p, miR30c-5p, miR30d-5p, and miR30e-5p, are downregulated in multiple myeloma plasma cells as compared with plasma cells from bone marrow of normal individuals (70, 71). We initially linked downregulation of this miRNA family with upregulation of BCL9 mRNA expression in patients with multiple myeloma, and discovered a possible mechanism that could account for high Wnt signaling in multiple myeloma cells (72). BCL9 is a critical transcriptional coactivator of the Wnt/β-catenin pathway, which is overactive in a large subset of patients with multiple myeloma and is believed to play a pivotal role in disease progression (14). We found that miR30-5p family targeting BCL9 by binding the 3’-UTR region of mRNA was downregulated in patients with multiple myeloma, which would contribute to upregulation of BCL9 protein and elevation of Wnt signaling in multiple myeloma cells (72). Overall, these studies increase the understanding of dysregulations of the Wnt pathway in multiple myeloma cells and provide firm pathologic support and a rationale for miR30-5p replacement therapy in patients with multiple myeloma by interfering Wnt pathway.

**miRNAs as Agents for Multiple Myeloma Therapy**

miRNAs have emerged as a powerful strategy for the suppression of gene expression, offering the potential for novel drugs, provided of course that the strategy can be implemented in vivo. The success of miRNA therapy necessarily relies on development of a suitable in vivo delivery system. Indeed, recent studies have yielded promising results in this regard.
Two different approaches exist for miRNA therapy. In the first, if the miRNA regulates expression of tumor suppressor genes, it can be used as a therapeutic target using "anti-miRs." In 2008, Santaris announced that it had commenced a clinical trial for SPC3649, an LNA-based antisense molecule against miR122-5p, for the treatment of hepatitis C. In multiple myeloma, by targeting miR21-5p in a preclinical study, it was shown that in vivo miR21-5p blockade inhibits tumor growth via upregulation of PTEN, Rho-B, and BTG2, providing a tempting rationale for the development of miR21-5p inhibitors as anti-multiple myeloma drugs (73). Furthermore, miR221-3p/miR222-3p inhibitors triggered in vitro antiproliferative effects and afforded significant antitumor activity in a xenograft model of multiple myeloma through upregulation of p27kip1, PUMA, PTEN, and p57kip2 (74). In the second approach, if the miRNA regulates expression of an oncoprotein, one can use replacement therapy or a "miR-mimic." It has been documented that aberrant DNA methyltransferase (DNMT) expression is efficiently modulated by synthetic miR29b-3p mimics (75), suggesting that this may be a novel approach to the development of miRNA-based therapy of multiple myeloma (76). Di Martino and colleagues were the first to prove that either transient expression of miR34a-5p using synthetic mimics or stable lentivirus-based enforced expression of an miR34a-5p gene triggers growth inhibition and apoptosis in multiple myeloma cells in vitro by targeting BCL2, CDK6, and NOTCH1 at both the mRNA and protein level (77). Importantly, synthetic miR34a-5p has been found to exert in vivo antitumor activity in clinically relevant murine models of human cancer (78). Very recently, Mirna Therapeutics presented interim phase I safety data in liver cancer for its lead product candidate MRX34 (miR34a-5p mimic) at AACR annual meeting (79).

In our recent study, an example of the second approach for miRNA therapy, it was shown that ectopic expression of miR30-5p was associated with decreased BCL9 expression, Wnt reporter activity, and expression of Wnt downstream target genes; as well as inhibition of proliferation and migration of multiple myeloma cells, along with promotion of apoptosis of multiple myeloma cells (72). In addition, enforced expression of miR30-5p was associated with decreased in the number of sorted multiple myeloma stem cells. These studies demonstrate that miR30s regulates the Wnt pathway by...
targeting BCL9 in multiple myeloma cells and therefore represents a promising novel therapeutic approach (Fig. 1). The potential promise for clinical translation of this approach was highlighted by the capacity of miR30-5p in replacement therapy using lipid nanoparticles to reduce tumor burden and metastasis, as well as enhance survival without the adverse effect of bone disease, in three murine preclinical models of human multiple myeloma. Although these studies indicate that miR30-5p replacement therapy is not associated with side effects on multiple myeloma-associated bone disease, these studies need further confirmation in cell-based functional assays using osteoblast cell lines. Some concern arose over the use of Wnt inhibitors for multiple myeloma treatment because of the role of the Wnt pathway in blocking osteoblast differentiation, and thus the possibility of enhancing bone disease. These reservations aside, miR30-5p treatment in multiple myeloma has emerged as a promising strategy for investigating and combating diseases of Wnt/β-catenin/BCL9 deregulation.

Conclusions and Future Directions

The Wnt/β-catenin/BCL9/B9L transcriptional complex is a novel and rational target for cancer therapy. Targeted inhibition of this nuclear complex selectively suppresses Wnt transcription and elicits mechanisms-based antitumor responses. The clinical translational potential of this approach is underscored by the capacity of stabilized α-helix peptides of BCL9 (11) and miR30-5p (72) to suppress growth, invasion, and metastasis in mouse xenograft models of multiple myeloma, and is further supported by the reported ability of carnosate to reduce the number of intestinal tumors in a mouse model of Wnt/β-catenin-driven cancer (39).

Potential approaches for targeting the Wnt/β-catenin/BCL9/B9L transcriptional complex include: disruption of β-catenin/BCL9/B9L interaction using stabilized α-helix peptides of BCL9 or small-molecule compounds and inhibition of BCL9 using miR30-5p. Next steps should include: exploring opportunities to further optimize stabilized α-helix peptides of BCL9 based on analysis of its pharmacokinetic and pharmacodynamic properties and in vivo efficacy in various Wnt-dependent cancer models; development of biochemical screening assays with which to perform structure–activity relationship (SAR) studies and identify small-molecule inhibitors of BCL9-HD2/β-catenin interaction; and investigating the role of miR30-5p in cancers other than multiple myeloma and to develop more efficient in vivo drug delivery systems with which to target multiple myeloma or other tumor cells. Indeed, the emergence of other peptide-based (11, 42) and small molecule (43) approaches to targeting the BCL9–β-catenin interface, as well as the development of cell-targeted therapies using aptamers (94), highlights the potential for clinical translation of this strategy for combating multiple myeloma and other Wnt-driven cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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