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MicroRNA-Specificity Protein (Sp) Transcription Factor Interactions and Significance in Carcinogenesis

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Abstract

Specificity protein (Sp) transcription factors (TFs) such as Sp1, Sp3 and Sp4 are overexpressed in tumors and Sp1 is a negative prognostic factor for multiple tumor types. Sp TFs regulate expression of pro-oncogenic factors important for cell proliferation, survival, angiogenesis, migration/invasion and inflammation and the high expression of Sp TFs in tumors is primarily due to miRNAs. For example, expression of tumor-suppressor-like miRNAs such as miR-200b/c, miR-335, miR-22, miR-149 and others that inactivate Sp1 expression is low in many tumor types. Research in our laboratory has also demonstrated that high expression of Sp TFs is also due to miRNA-dependent inhibition of the transcriptional repressors ZBTB10 and ZBTB4 by miR-27a and miR-20a/miR-17p, respectively. Thus, miRNAs play a critical role in maintaining high levels of Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated genes in tumors and cancer cells, and there is ample evidence that anticancer agents targeting the miRNASp TF axis can be highly effective for cancer chemotherapy.

Keywords

specificity protein transcription factors (Sp TFs); Sp1; Sp3; Sp4; miRNAs inhibit Sp TFs; miR-27a:ZBT10; miR-20a/miR-17-5p:ZBTB4; Sp TFs regulation

INTRODUCTION

With the advent of high throughput genome sequencing and annotation technologies, the increasing significance of non-protein encoding RNAs (ncRNAs) in cellular homeostasis and disease has become apparent (1-9). Long non-coding RNAs (lncRNAs) contain >200 nucleotides and recent reports from the ENCODE Project Consortium have identified 9277 lncRNA genes that produce 14,880 transcripts (10). The biological roles of only a small fraction of lncRNAs have been reported but it is apparent that they exhibit multiple functions and contribute to cellular homeostasis and diseases including cancer (3-5). LncRNAs can interact with RNA, DNA and proteins, and their mechanisms of action

Conflict of Interest

Stephen Safe declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by the author.

include their activity as decoys and guides that titrate away or guide proteins to their presumptive DNA targets, respectively (3, 4). MicroRNAs (miRNAs, miRs) are small non-coding RNAs containing 21-23 nucleotides that primarily repress gene expression, and it has been estimated that there are in excess of 2500 human miRNAs that regulate expression and the related functions of over 50% of the human genome (8). Among non-coding RNAs, miRNAs have been most extensively investigated and their regulation and function in maintaining cellular homeostasis and their role in diseases, particularly cancer have been extensively investigated (6-8). For example, miR-143/145 exhibit tumor suppressor-like activity and are downregulated in multiple tumors. Mice with deletion of miR-143/145 exhibit defective epithelial regeneration after injury and this was linked to de-repression a critical miRNA target insulin-like growth factor binding protein 5 (11). MiR-21 has been extensively identified as an oncogenic miRNA and in mouse models, it has been shown that overexpression or knockdown of the miR-21 enhances or inhibits lung tumorigenesis, respectively (12).

MiRNA interactions with target mRNAs are dependent on specific base pair interactions of miRNAs with complementary 3'-UTR sequences in targeted mRNAs. These interactions usually involved interactions of ≥6 seed sequence oligonucleotides of the miRNA with complementary 3'-UTRs of targeted mRNAs, and several computational methods have been used to predict mRNA targets and the ranking of potential targets. The major problem associated with identification and characterization of a miRNA that regulates a specific mRNA and its resulting gene product is that most miRNAs can potentially target ≥300 mRNAs [reviewed in (8)]. Moreover, every gene can potentially be regulated by multiple miRNAs. Thus, the common cellular approaches such as miRNA overexpression and knockdown used to study the targets and function of miRNAs must be interpreted cautiously due to modulation of more than one pathway that could affect multiple genes/pathways and functions.

Regulation of Specificity Proteins (Sp) Transcription Factors by miRNAs in Cancer

Sp transcription factors (TFs) and cancer—Sp TFs Sp1, Sp3 and Sp4 are highly expressed in most cancer cells/tumors (13-20), and Sp1 is a negative prognostic factor for survival of glioma, pancreatic and gastric cancer patients (21-24). Although Sp1 and other Sp proteins are important for early embryonic and postnatal development in mice, their expression is relatively low in adult tissue, and there is evidence that Sp1 expression decreases with age in rodents and humans (25-27). The functional importance of Sp1, Sp3 and Sp4 in cancer cells has been confirmed by RNAi showing that knockdown (singly or combined) decreases cell proliferation, survival, angiogenesis and inflammation. These results are consistent with identification (by RNAi) of several pro-oncogenic Sp-regulated genes important for cell growth (cyclin D1, EGFR, c-Met), survival (bcl-2, survivin), angiogenesis (VEGF, VEGF receptors [VEGFR]) and inflammation (p65-NF[.kappa]B) (13-20). Thus, Sp TFs clearly contribute to the transformed cell phenotype and represent an example of non-oncogene addiction by cancer cells (28).

Regulation of Sp TFs by miRNAs: inhibition—Since Sp1 and other Sp TFs (e.g. Sp3 and Sp4) are overexpressed in cancer cells and exhibit pro-oncogenic activities, miRNAs

that interact with and suppress expression of these genes tend to exhibit tumor suppressor-like activities. Sp1 is overexpressed in gastric tumors and is a negative prognostic factor for patient survival (22, 23) and several studies show that miRNAs are important for regulation of Sp1 in gastric cancer cells (29-32). For example, miR-200b and miR-200c are downregulated in gastric tumors and cancer cells, and higher levels of these miRs correlate with a good prognosis for patients (29). Ectopic expression of miR-200b and miR-200c in gastric cancer cells inhibit their growth and invasion and these miRs target Sp1 and DNA methyltransferases. MiR-335 expression also decreases during tumor development and formation (33-36), and low levels are detected in gastric cancer cell lines and low expression in tumors are associated with lymph node metastasis and other negative prognostic factors (30). MiR-335 overexpression in gastric cell lines results in decreased proliferation and invasion, and both Sp1 and bcl-2 have been identified as targets of miR-335 (30). MiR-22 expression is also low in gastric tumors compared to non-tumor tissue; miR-22 expression inhibits gastric cancer cell invasion and proliferation and Sp1 is a direct target of miR-22 (31). Moreover, there is an inverse linear correlation between expression of miR-22 and Sp1 mRNA in gastric tumors. A recent study further extended the inhibitory miR-Sp1 interaction in gastric cancers by identifying miR-145, miR-133a and miR-133b as miRs that also target Sp1 in gastric cancer cell lines (32). Results of overexpression studies showed that this set of miRs also inhibits gastric cancer cell proliferation and invasion and this is due, in part, to downregulation of Sp1-regulated genes such as matrix metalloproteainase-9 and cyclin D1.

MiR-429 expression is lower in esophageal tumors than in non-tumor tissue and low expression is significantly associated with increased lymph node metastasis (37). Overexpression of miR-429 in esophageal cancer cell lines increases apoptosis and decreases migration, and miR-249 also binds 3'-UTR regions of both Sp1 and bcl-2 (37). MiR-29b interacts with Sp1 in both acute myeloid leukemia and multiple myeloma cells (38-40). Sp1 and NFκB activate the KIT gene in acute myeloid leukemia cells which in turn results in MYC-dependent repression of miR-29b. Decreased expression of miR-29b results in increased levels of Sp1 since miR-29b targets (downregulates Sp1) (38). In addition, Sp/HDAC/NFκB also inhibits miR-29b expression and this complex regulatory system that drives KIT expression can be targeted *in vivo* by overexpression of miR-29b (as a nanoparticle complex) which results in a marked decrease in expression of Sp1 and other miR-29b targets (39). Sp1 is also a negative regulator of miR-29b in multiple myeloma cells but miR-29b expression also decreases Sp1 protein in these cells (40), suggesting a regulator loop similar to that observed in acute myeloid leukemia cells.

MiR-375 expression is decreased in cervical tumors compared to non-tumor tissue and low expression in tumors is a negative prognostic factor for cervical cancer patients (41). Overexpression of miR-375 decreases cervical cancer cell proliferation, migration and invasion and downregulates Sp1 (protein) expression, and Sp1 knockdown and miR-375 overexpression results in similar functional responses (41). Similar studies in prostate cancer cells with miR-330 (42) and colon cancer cells (miR-149) (43) showed that both miRs exhibit tumor suppressor-like activity (inhibition of cell growth and invasion) and downregulates Sp1. CD147 is overexpressed in breast tumors and is associated with tumor progression and this gene is regulated by Sp1 and cMyc. MiR-22 decreases CD147

expression through targeting Sp1 and the tumor suppressor-like activity of miR-22 is due, in part, to downregulation of Sp1 (44).

These results as illustrated in Figure 1A clearly demonstrate that miRNAs play a critical role in regulating Sp1 expression in several cancer cell types and corresponding tumors and suggest that the high expression and pro-oncogenic functions of Sp1 in multiple cancers are due, in part, to decreased expression of tumor suppressor-like miRNAs that target Sp1. The inverse relationship between levels of miRNAs 200b, 200c, 335, 22, 145, 133a, 133b, 429, 29b, 375, 330 and 149 and Sp1 suggests that drugs targeting Sp1 or inducing these miRs may be important therapeutic avenues for cancer chemotherapy. All previous studies have focused primarily on Sp1; however, there is evidence that in some cancer cells that Sp3 and Sp4 have similar prooncogenic activities that should also be considered and further investigated (45).

Indirect regulation of Sp TFs by miRNAs—Studies in this laboratory reported that Sp1, Sp3 and Sp4 are overexpressed in breast cancer cell lines compared to non-transformed mammary cells and the underlying mechanisms associated with high expression of Sp TFs were investigated (46). It was previously reported that miR-27a suppresses expression of ZBTB10 (47) which is a member of the POK family of transcriptional repressors (48, 49) and overexpression of ZBTB10 decreased expression of the Sp-regulated gastrin gene (50). Since miR-27a and associated members of the miR-23a ~ miR-27a ~ miR-24-2 cluster are overexpressed in multiple cancer cell lines and tumors (46, 51-53), we further investigated the role of miR-27a-mediated suppression of ZBTB10 as a mechanism for maintaining high levels of Sp1, Sp3 and Sp4 in breast cancer cells (46). Transfection of breast cancer cells with miR-27a antagomirs increased expression of ZBTB10 and decreased levels of Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated gene products and similar results were observed after overexpression of ZBTB10 in breast cancer cell lines. Not surprisingly, miR-27 antagomirs also induced apoptosis, inhibited breast cancer cell growth and cell cycle progression and this was due not only to ZBTB10-mediated repression of Sp TFs and Sp-regulated genes but also activation of Myt-1 which inhibited cells at the G2/M phase of the cell cycle. The role of miR-27a:ZBTB10 in the maintenance of high levels of Sp1, Sp3 and Sp4 has been observed in breast, pancreatic, colon, rhabdomyosarcoma and bladder cancer cell lines (13-15, 46, 51-61).

We also examined breast cancer patient array data bases for expression of ZBTB family members and identified ZBTB4 as a prognostic factor in which high or low expression predicts increased or decreased relapse-free survival, respectively (62). The NCI-60 cell mRNA and miRNA data sets were used to examine possible inverse correlations between ZBTB4 expression and miRs and several miRNAs that could potentially target ZBTB4 were identified. These included members of miR-17-92 (miR-20a and miR-17-5p) and miR-106b-25 (miR-106 and miR-93) clusters which have identical seed sequences. Subsequent studies showed that ZBTB4 overexpression or miR antagomirs decreased expression of Sp1, Sp3, Sp4 and Sp-regulated genes in breast bladder and prostate cancer cells (57, 62, 63), confirming that miR-20a and paralogs suppress ZBTB4 and this also contributes to the high expression of Sp TFs in cancer cell lines. A recent study also showed that miR-20a/miR-17-5p-mediated suppression of ZBTB4 also contributed to high Sp

expression in pancreatic cancer cells and ZBTB4 expression also downregulated Sp1, Sp3 and Sp4 (64). These studies demonstrate a critical role for miR:ZBTB interactions in maintaining high levels of Sp proteins and pro-oncogenic Sp-regulated genes in cancer cell lines (Fig. 1B) and therefore these miRs are potential drug targets for cancer chemotherapy.

Drugs that target miR:ZBTB decrease Sp TFs—The list of anticancer agents that downregulate Sp1, Sp3 and Sp4 is continually expanding and includes clinically used compounds such as arsenic trioxide, bortezomib, metformin, several non-steroidal anti-inflammatory drugs (NSAIDs) including aspirin and tolfenamic acid, curcumin, naturally-occurring and synthetic triterpenoids, other natural products, and reactive oxygen species (ROS)-inducing compounds (13-20, 54-69) . Our studies show that Sp proteins are downregulated via two major pathways, namely Sp protein degradation (proteasomes and caspases) and repression of Sp1, Sp3 and Sp4 gene expression. At least one of the gene repression pathways has been characterized and this explains the mechanism of ROS-mediated downregulation of Sp1, Sp3 and Sp4 (Fig. 1B) (45). A recent study reported that hydrogen peroxide rapidly decreased expression of cMYC and several other genes in colon cancer cells and this was due to genome-wide shifts of chromatin modifying repressor complexes from non-GC-rich to GC-rich sites (70). Studies in this laboratory have now demonstrated that ROS inducers such as phenethylisothiocyanate (PEITC) rapidly decrease cMYC expression in pancreatic cancer cells, and cMYC regulates both miR-27a and miR-20a/miR-17-5p expression which is also decreased in pancreatic cancer cells by PEITC (64). Moreover, knockdown of cMYC by RNA interference decreases expression of miR-27a and miR-20a/17-5p, induces ZBTB10 and ZBTB4, and downregulates Sp1, Sp3, Sp4 and prooncogenic Sp-regulated genes. These results clearly demonstrate that another important function of MYC in cancer cells is the maintenance of high levels of Sp TFs due to regulation of miRs that in turn inhibit expression of the Sp-repressors ZBTB10 and ZBTB4.

Sp regulation of miRs—Since Sp TFs regulate multiple genes in breast cancer cells, it is not surprising that they also regulate expression of miRNAs. For example, the miR-200b ~ 200a ~ 429 minimal promoter contains several GC-rich Sp binding sites that are important for high expression of this miRNA cluster in breast cancer cells, and knockdown of Sp1 by RNAi decreases expression of members of this miRNA cluster (71). There is also evidence that Sp1 regulates expression of the pro-oncogenic miR-17-92 cluster in cancer cell lines and Sp1 plays a role in expression of miR-365 which in turn suppresses interleukin 6 (72, 73). Ongoing studies in this laboratory indicate that Sp1, Sp3 and Sp4 regulate other miRNAs and long non-coding RNAs, suggesting that these non-coding RNAs are also potential targets for agents that downregulate Sp TFs.

Conclusions

MiRNAs play an integral role in maintaining cellular homeostasis and in disease, and this is particularly true in cancer. MiRs exhibit both oncogenic and tumor suppressor-like activity and there is great potential for using miRNAs as direct drug targets. Serum levels of miRs are emerging as potential prognostic factors and potential indicators of drug efficacy, and drug-induced downregulation of miRs could be observed in serum and be a marker of

treatment efficacy. An important functional component of miRs is their linkage to the direct and indirect regulation of Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated genes in cancer cells and tumors. Sp TFs are prototypical examples of non-oncogene addition by cancer cells, and development of ROS-inducing anticancer agents that target these factors is also dependent, in part, on miRs, particularly those that are regulated by cMYC (74, 75).

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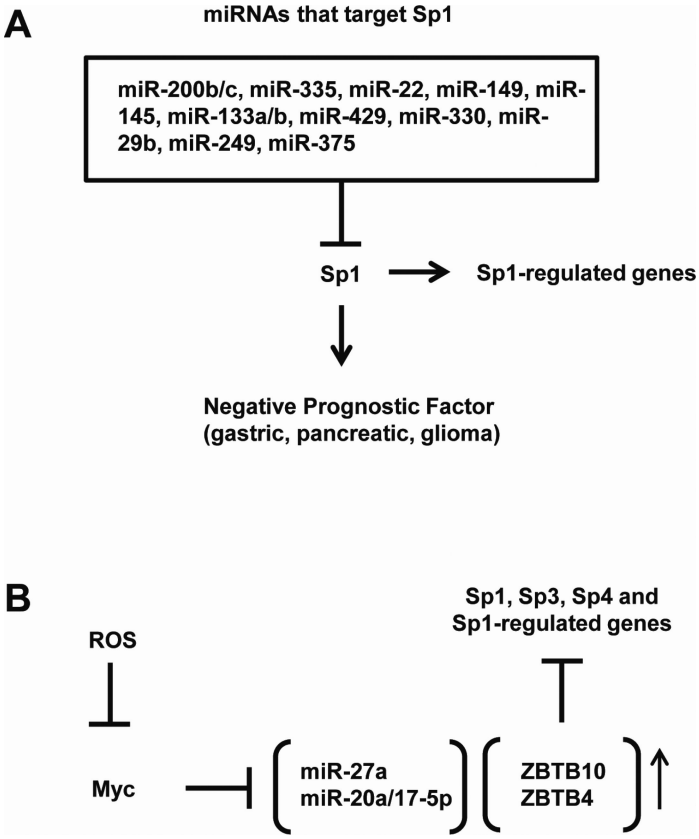

Figure 1.

(A) Sp1 levels are high in solid tumors due to the low expression of most miRNAs that suppress Sp1. (B) ROS decreases expression of Myc and Myc-related miRs to induce expression of Sp repressors (ZBTB10/ZBTB4) that downregulate Sp1, Sp3, Sp4, and Sp-regulated genes (64).