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The miR-200 and miR-221/222 microRNA Families: Opposing Effects on Epithelial Identity

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Abstract

Carcinogenesis is a complex process during which cells undergo genetic and epigenetic alterations. These changes can lead tumor cells to acquire characteristics that enable movement from the primary site of origin when conditions become unfavorable. Such characteristics include gain of front-rear polarity, increased migration/invasion, and resistance to anoikis, which facilitate tumor survival during metastasis. An epithelial to mesenchymal transition (EMT) constitutes one way that cancer cells can gain traits that promote tumor progression and metastasis. Two microRNA (miRNA) families, the miR-200 and miR-221 families, play crucial opposing roles that affect the differentiation state of breast cancers. These two families are differentially expressed between the luminal A subtype of breast cancer as compared to the less well-differentiated triple negative breast cancers (TNBCs) that exhibit markers indicative of an EMT. The miR-200 family promotes a well-differentiated epithelial phenotype, while high miR-221/222 results in a poorly differentiated, mesenchymal-like phenotype. This review focuses on the mechanisms (specific proven targets) by which these two miRNA families exert opposing effects on cellular plasticity during breast tumorigenesis and metastasis.

Keywords

miR-200; miR-221; miR-222; EMT; MET; breast cancer

Introduction

miRNAs are small (18–25 nucleotide) non-coding RNAs that regulate gene expression post-transcriptionally by binding to the 3’ untranslated region (UTR) of target messenger RNAs (mRNAs) (1), and inhibiting translation or targeting the mRNA for degradation (2). The extent to which miRNAs regulate the human transcriptome is still under investigation; however, miRNAs can target hundreds of genes, suggesting that their regulatory role may be as significant as that of transcription factors. miRNAs are differentially regulated during

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development (3–5). Controlled epithelial to mesenchymal transition (EMT) is a normal process in development, required for processes such as gastrulation, mammary gland branching, and neural crest formation (reviewed in (6)). However, EMT is a pathological event in cancer that contributes to the gain of aggressive characteristics that facilitate metastasis (7–10). In cancer EMT, carcinoma cells do not become mesenchymal cells, although there can be a marked loss of epithelial hallmarks and a shift toward mesenchymal and even neuronal gene expression. It is widely believed that acquisition of these characteristics can allow tumor cells to become motile, invasive, and able to intravasate into the blood and lymph vessels and survive the metastatic journey. Transcription factors, such as Twist, Snai1, and ZEB1/2 (Reviewed in (11)) regulate both normal and oncogenic EMT. ZEB1 (zinc finger E-box binding homeobox 1) and ZEB2 (also known as SIP1) directly repress the adherens junction protein E-cadherin (12–14) and other genes involved in polarity and epithelial identity (15,16).

ZEB1/2 are post-transcriptionally controlled by the miR-200 family of miRNAs (17–19), and ZEB2 is indirectly controlled by the miR-221 family (20). Indeed, recent studies have identified the miR-200 and miR-221 families as differentially expressed in carcinomas, particularly in breast cancer (20,21). Specifically, the miR-200 family is high in the luminal breast cancer subtypes, while miR-221/222 is overexpressed in triple negative breast cancers (TNBCs), particularly those that have undergone EMT. These miRNAs control expression of many genes that define the EMT-like phenotype and likely affect tumor behavior and clinical outcome by influencing metastatic potential. Thus, in this review we focus on the opposing roles of these two miRNA families in controlling differentiation state or epithelial identity in breast cancer.

miR-200 protection of the epithelial phenotype

miR-200 family regulation of EMT in breast cancer

The miR-200 family of miRNAs is comprised of two polycistronic clusters – miR-200c and miR-141 on chromosome 12 and miR-200b, miR-200a and miR-429 on chromosome 1. miR-200a and miR-141 share a seed sequence, while miR-200b, miR-200c and miR-429 also share a seed sequence, which differs from that of miR-200a/141 by one nucleotide. Because of their sequence similarity, the miRNAs are predicted to share gene targets; however, there is evidence that the two clusters control different regulatory networks even in the same model. In MDA-231 cells the miR-200bc/429 cluster induces G2/M arrest, while miR-200a/141 induces G0/1 arrest (22). Additionally, miR-200c directly targets and down-regulates the transcription factor ZEB1, while miR-200a does not (23).

The miR-200 family was first discovered to directly target and down-regulate the E-cadherin transcriptional repressors ZEB1 and ZEB2, leading to restoration of an epithelial phenotype in breast cancer cell lines, characterized by an increase in E-cadherin expression, and decreased migration and invasion (17–19). Expression of the miR-200 family correlates with an epithelial-like phenotype in the National Cancer Institute (NCI) panel of 60 cancer cells lines (19), and suppresses EMT in several additional cancer models, including bladder (24), colorectal (25,26), and lung (27–30). Although genes encoding ZEB1/2 are the best-studied targets of the miR-200 family, the small consensus binding sequence of miRNAs results in

many bioinformatically predicted targets. The miR-200 family has now been confirmed to directly target other genes involved in various aspects of EMT. One aspect of EMT that has been particularly well studied is the increase in migratory and invasive capacity. Targeting and repression of the genes encoding ZEB1/2 by miR-200c and the resultant increase in E-cadherin decreases migration and invasion; however, direct targeting of genes encoding the actin cytoskeleton associated proteins WAVE3 (31) and MSN (32), and the extracellular matrix component FN1 (32) also contribute to suppression of motility and invasion. The miR-200 family also targets two genes involved in cell cycle control, RND3 (33) and FOG2 (34).

The power of miRNAs lies in their ability to target multiple genes that contribute to a pathway or phenotype. For instance, normal well-differentiated mammary epithelial cells exhibit hallmarks such as E-cadherin and hormone receptor expression, while poorly differentiated breast carcinoma cells loose these characteristics. When carcinoma cells revert towards a less-differentiated state, in addition to loosing expression of epithelial hallmarks, they also inappropriately gain expression of proteins that confer the ability to move away from the primary tumor when conditions are harsh (hypoxia, lack of nutrients, and build-up of waste products). The tumor cells must also be able to resist anoikis in order to survive detachment from the basement membrane.

Anoikis resistance is a relatively poorly understood and understudied aspect of EMT. Anoikis is apoptosis induced when cells lose attachment to their native extracellular matrix (ECM), and resistance to anoikis is required for cancer cells to survive as they move away from the primary tumor, and travel through the vasculature or lymphatics to metastatic sites. Data from our lab demonstrate that miR-200c suppresses anoikis resistance through direct targeting of *NTRK2*, the gene encoding TrkB (32), a receptor tyrosine kinase involved in neuronal development and differentiation. TrkB was first associated with anoikis resistance when it was isolated from a cDNA library screen designed to identify genes capable of conferring anoikis resistance to normal intestinal epithelial cells (35). TrkB is involved in anoikis resistance in breast cancer (32,35–38) and is specifically expressed in TNBCs that have undergone EMT, but not luminal A lines (32).

Resistance to chemotherapy is a critical aspect of tumorigenesis also associated with acquisition of an EMT phenotype. The miR-200 family has been found to be involved in maintaining sensitivity to two classes of chemotherapeutics to date, microtubule targeting agents, and DNA damaging drugs. In aggressive cancer cells resistant to taxanes, restoration of miR-200c increases sensitivity due to its direct targeting of *TUBB3*, the gene encoding class III beta tubulin (39,40). TUBB3 is a tubulin isoform aberrantly expressed in several types of carcinomas (41–43), including breast (44,45), that leads to resistance to taxanes (Reviewed in (46)). Additionally, the miR-200 family is down-regulated in MCF7 cells selected for resistance to cisplatin (47), or doxorubicin (48). Indeed, miR-200 expression correlates with sensitivity to EGFR blocking agents in bladder cancer, and restoration of miR-200 family members increased sensitivity to EGFR inhibitors in mesenchymal-like cell lines (49). Additionally, lower expression of miR-200c was observed in a panel of 39 breast cancer patients resistant to chemotherapy (48). The authors speculate that these effects may be due to the predicted targeting of the multidrug resistance gene 1 by miR-200c, but this

remains to be proven. Finally, miR-200c directly targets FAP-1, leading to restoration of sensitivity to CD-95 (Fas) -mediated apoptosis (50). Thus, the miR-200 family exerts multi-level control over apoptosis in epithelial cells. The family promotes sensitivity to natural apoptotic stimuli, including loss of adhesion and Fas signaling, while also preventing resistance to several classes of therapeutic agents.

While not classically thought of as a characteristic of EMT, an overall decrease in miRNA abundance is found in aggressive cancer cells (51,52). Dicer, an enzyme involved in the maturation of miRNAs, is often low in cancers that have undergone EMT (53). While the mechanism remains to be elucidated, we demonstrated that restoration of miR-200c to TNBC cell lines causes an increase in Dicer protein (21). Since relatively high levels of Dicer and overall miRNA abundance are characteristic of normal epithelial cells, this is a unique mechanism through which the miR-200 family promotes an epithelial phenotype.

In addition to regulation of EMT, there is emerging evidence that the miR-200 family plays a role in epigenetic regulation and inhibition of stem cell-like qualities in breast, prostate (54,55), and colorectal cancer cells (26). Expression of both miR-200 family clusters is down-regulated in stem cells isolated from normal human breast, and murine mammary glands, as well as in stem cells isolated from breast cancer patients (56). Inhibition of miR-200 leads to an enrichment of the stem cell population, and up-regulation of the miR-200b direct target Suz12, a subunit of the polycomb repressor complex. Increased Suz12 leads to trimethylation and polycomb-mediated repression of the E-cadherin promoter (57). Another direct target, the gene encoding class III histone deacetylase, SIRT1, deacetylates histone H3 at the E-cadherin promoter, and miR-200 mediated repression of SIRT further relieves repression of E-cadherin (58). The miR-200 family also directly targets and represses *Bmi1*, allowing further repression of stemness (26). Additionally, expression of miR-200c inhibits clonal expansion of stem cells, and prevents tumor formation from patient-derived breast cancer stem cells transplanted into mice (56). Finally, two important stem cell factors, Sox2 and KLF4 have been found to be down-regulated following restoration of miR-200c (26). Thus, the miR-200 family controls multiple genes that repress cancer stem cells, leading to restoration of an epithelial phenotype and decreased aggressiveness. The genes and aggressive phenotypes repressed by the miR-200 family are detailed in Figure 1.

The miR-200 family is highly expressed in luminal A breast cancer cell lines and lost in TNBC lines (21); however, data from primary and metastatic breast cancer samples are not as clear. Based on the cell line data, it was expected that the miR-200 family would be down-regulated in aggressive tumors and metastases. While this is true in some models, and restoration of miR-200 to a TNBC cell line prevents metastases (59), in other models the miR-200 family positively correlates with metastases (60,61). Consistent with the theory that miR-200c positively correlates with a well-differentiated phenotype, the miR-200 family is very low in the poorly differentiated claudin-low subtype of breast cancer , while expression of ZEB1/2, vimentin, and Twist are high and these tumors are enriched for tumor initiating cells, suggesting that the miR-200 family must be down-regulated for formation of an aggressive subpopulation of tumor cells (62). However, while several profiling studies found that expression of the miR-200 family is lost between normal breast tissue and

malignant breast cancers (18,63) one profiling experiment (64), comparing luminal A, luminal B, basal-like and malignant myoepithelioma, revealed that while the miR-200 family is highly expressed in luminal tumors, it is also highly expressed in basal-like tumors. Only malignant myoepitheliomas showed down-regulation of the miR-200 family, which is consistent with a strong EMT phenotype (64).

Expression of the miR-200 family in metastatic disease has been even more contested. While one group found the miR-200 family to be down-regulated between matched primary versus metastatic breast, colon, lung and bladder cancers (65), another showed that the miR-200 family is over-expressed in matched metastases, and that higher than median expression of several family members correlates with decreased progression free survival in estrogen receptor (ER) positive breast tumors (61). In contrast, high expression of miR-200b, and low expression of Suz12 can distinguish primary breast tumors from metastases, which express low miR-200b and high Suz12 (57). Further complicating the matter are two studies performed in syngeneic mouse mammary carcinoma models. In one study, using the 4T1 panel of cells lines, expression of miR-200 in a non-metastatic cell line increased metastasis (60). Forced expression of miR-200c and miR-141, or all members of the miR-200 family led to increased metastasis in a similar model, the 4TO7 cell line (61). These studies suggest that expression of the miR-200 family may induce mesenchymal to epithelial transition (MET) during the metastatic cascade. Induction of MET may be necessary for colonization of cells at the metastatic site, which would be consistent with increased expression of the miR-200 family. It is also possible that EMT is not required for metastasis in these models. Another possible explanation is that there are differences in the rate limiting steps of the metastatic cascade across models, which could affect the necessity of MET in colonization. Finally, regulated expression of miR-200 may be important for phenotypic plasticity, and may allow cells to transition between epithelial and mesenchymal states as needed.

miR-200 family in plasticity

There is mounting evidence that both EMT and MET are important in the progression of carcinomas, and that carcinoma cells exhibit increased plasticity, allowing them to transition as necessary. Both EMT and MET are required for proper development, and the role of the miR-200 family in transitions between the epithelial and mesenchymal states is becoming clear. During embryonic stem cell differentiation, the miR-200 family is down-regulated by Snai1 and Wnt signaling, and forced expression of miR-200 leads to cells stalling at the epiblast-like stem cell stage of differentiation (66). The miR-200 family is also regulated by c-Myc in differentiating embryonic stem cells (67).

Forced expression of miR-200c in epithelial cells of the developing mammary gland suppresses ductal growth (56), suggesting that plasticity is required for proper formation of the ducts. Similarly, forced expression of miR-200 in plastic, metastatic lung adenocarcinoma cells reversed plasticity, preventing the cells from undergoing EMT or metastasizing (68). Manipulation of ZEB1/2 and the miR-200 family in Madin-Darby canine kidney (MDCK) cells leads to EMT and MET, respectively, but the states remain plastic and can be reversed (69). miRNA profiling of embryonic stem cells, induced pluripotent stem

(iPSC) cells, differentiated cells and cancer cells revealed that the pluripotent stem cells formed two clusters, irrespective of the origin of the cells (embryonic versus induced). The miRNAs that distinguished these groups also differentiated normal cells from cancer cells. Expression of miR-92 or miR-200 family members in iPSCs changed their classification status, leading the authors to suggest that the subdivision in pluripotent stem cell states does not reflect their origin, but rather miRNA and gene expression network (70). Similarly, the miR-200 family is regulated during reprogramming of somatic cells into iPSCs (71). Thus, the miR-200 family, as well as EMT-inducing transcription factors, must be expressed in the proper order to allow differentiation of embryonic stem cells.

Regulation of the miR-200 family

The most potent regulators of the miR-200 family are ZEB1 and ZEB2, which have been demonstrated to target E-boxes in the miR-200 cluster promoters (72,73). Another well recognized EMT inducer, transforming growth factor beta (TGF-β), has also been shown to reduce expression of the miR-200 family in transformed human breast epithelial cells (74), murine mammary epithelial cells (75), prostate cancer cells (76), and canine renal MDCK cells, a model of the epithelial phenotype (18,77). Indeed, treatment with TGF-β leads to hypermethylation of the miR-200 promoters, potentially through miR-200a-mediated direct targeting of the histone deacetylase SIRT1 (74). Further study of the role of epigenetic regulation of the family revealed that the promoters are unmethylated in epithelial cells, and in cancer cells that express the family, but heavily methylated in fibroblasts and tumors that do not express the miR-200 family (78,79). Furthermore, the permissive epigenetic mark, histone H3 acetylation, is decreased at the miR-200 promoter in cancer cells lacking expression of the family (80), an epigenetic mark potentially influenced by miR-200a direct targeting of HDAC4. Together, this data indicates that while classical EMT-inducers control expression of the miR-200 family in tumorigenesis, epigenetic control is also important, and potentially forms feedback loops through miR-200 control of epigenetic regulators, including SIRT1, HDAC4, and Suz12.

Several other EMT inducers down-regulate the miR-200 family, including platelet derived growth factor (PDGF) (81), long-term treatment with the epidermal growth factor receptor (EGFR) inhibitor gemcitabine (82), and carcinogen induced tumorigenesis (83). Interestingly, treatment of pancreatic cancer cells with curcumin, or the analog CDF, along with gemcitabine lead to increased miR-200 family expression (81,84). Additionally, Akt isoforms leads to differential miRNA expression profiles. Expression of only Akt2 dramatically decreases expression of the miR-200 family, while knockdown of Akt1 induced EMT by reducing expression of the miR-200 family. The authors suggest that the expression of miR-200 family members depends on the ratio of Akt1/Akt2, rather than the overall activity of Akt (85). To date, the only known activators of miR-200 expression are the tumor suppressors p53 (86,87), p63, and p73 (88), and ERalpha (89). However, there are likely other positive-regulators of the miR-200 family.

miR-221/222 suppression of the epithelial phenotype

miR-221/222 expression in breast cancer and other carcinomas

miR-221 and miR-222 are found on the X chromosome and are expressed from a single transcript. For many cancer types, miR-221/222 are considered oncomiRs, and are overexpressed in tumor compared to normal tissue of origin. This expression pattern holds true in breast (63), prostate (90), gastric (91), bladder (92), papillary thyroid carcinoma (93), colorectal cancer (94), melanoma (95), and acute myeloid leukemia (96). High miR-221/222 expression is associated with increased tumor grade (97,98) and poor prognosis (99). High miR-221 is found in prostate cancer cell lines, where it is associated with aggressive phenotypes, such as androgen-independence and neuroendocrine differentiation (90).

Several studies have demonstrated that miR-221/222 directly target ERα (21,100,101). In breast cancer, miR-221/222 negatively correlate with ER status, and are more highly expressed in triple negative cell lines as compared to luminal (20,21,100) and the same holds true in clinical samples (21,102). Additionally, in the murine mammary tumor virus (MMTV)-c-myc mouse model of mammary carcinoma, miR-222 is increased during tumorigenesis (103). However, some controversy exists, since one study observed that although miR-221 is overexpressed in TNBCs and is associated with poor disease-free and overall survival, there was no difference in miR-222 expression between breast cancer and normal epithelial tissue (99). Additionally, another study found that miR-221 expression positively correlated with ER status in breast cancer patient samples, while miR-222 expression did not change between ER positive and ER negative samples (104). Thus, as with the miR-200 family, although expression of miR-221/222 correlates strongly with specific phenotypes *in vitro* in breast cancer cell lines, more work is required to fully elucidate the role of the family in human tumors.

miR-221/222 in EMT and metastasis

Since miR-221/222 are often overexpressed in poorly differentiated, aggressive cancers, it stands to reason that these miRNAs play an active role in promoting EMT. Increasing miR-221 or 222 can affect various characteristics associated with EMT, including increased invasive capacity (90,105), and anoikis resistance (106). Low Dicer is characteristic of poorly differentiated cells and cells that have undergone EMT. In TNBC lines, miR-221/222 directly target and repress Dicer1 (21), leading to the possibility that aberrant expression of miR-221/222 leads to decreased Dicer, which in turn leads to a decrease in overall miRNA abundance.

Long term mammosphere culture of MCF7 cells induces EMT, with the resulting cells displaying a basal B phenotype (107). The cells also exhibit increased expression of stem cell markers (CD44 + /CD24 − /low), and exhibited stem cell-like characteristics, including chemoresistance. qRT-PCR miRNA profiling demonstrates that miR-200c, −203 and 205 are decreased, while miR-221/222 are increased in the mammosphere cultured cells, with miR-222 increased 20-fold (107). Thus, although further more exhaustive and rigorous genetic analysis of necessity and sufficiency remains to be performed, it appears that induction of EMT in luminal breast cancer cells involves decreased expression of the

miR-200 family and increased expression of miR-221/222. Although miR-221/222 are high in both basal A and B breast cancer, their expression is higher in the basal B subtype, which has a more mesenchymal phenotype (20), consistent with the role of miR-221/222 in EMT. Forced expression of miR-221/222 in luminal breast cancer cells causes a decrease in E-cadherin and an increase in the mesenchymal marker vimentin (20). Luminal cells expressing miR-221/222 gained a more mesenchymal morphology and had increased migratory and invasive capacity. Conversely, inhibition of miR-221/222 in basal-like cells promoted MET (108). miR-221/222 promote a mesenchymal phenotype in part by directly targeting trichorhinophalangeal 1 (TRPS1), and keeping its levels low (20). TRPS1 is a transcriptional repressor that binds to GATA sites that can promote MET (20), and is underexpressed in breast cancers with poor clinical outcome (109). TRPS1 represses the mesenchymal transcription factor ZEB2 through a GATA site in its promoter. As ZEB2 is a repressor of E-cadherin, this provides a functional link between expression of miR-221/222 and repression of E-cadherin in basal breast cancers (20,110).

miR-221/222 control of proliferation

miR-221/222 positively influence cellular proliferation in many types of cancers. While there are several mechanisms through which increased growth rate is achieved, the best studied is direct targeting of $p27^{KIP1}$ (98,111), and $p57^{KIP2}$ (112,113). In patient samples, miR-221 or miR-222 levels are often inversely correlated with $p27^{KIP1}$ (111,114–116) or $p57^{KIP2}$ (94,112). Increasing the expression of miR-221 or miR-222 causes increased proliferation *in vitro* (111,114), and increased tumor growth in xenograft tumor models (117). Conversely, antagonizing miR-221/222 results in decreased proliferation both *in vitro* (94) and *in vivo* (118). In one study, decreased tumor growth was achieved through *in vivo* administration of cholesterol modified anti-miR-221, which suggests that miR-221 can be a viable therapeutic target for the treatment of aggressive cancers (119).

Direct targets other than $p27^{KIP1}$ and $p57^{KIP2}$ can also mediate the proliferative effects of miR-221/222. In gastric cancer cells, the proliferative effects of miR-221/222 are partially due to their ability to directly target PTEN (105), and targeting of PTEN is also likely to play an important role in breast carcinomas. Additionally, miR-221/222 directly target ARH1 (120), a tumor suppressor protein decreased in many types of cancers (121–123). Loss of ARH1 results in increased proliferation, colony formation and invasion (120). Thus, miR-221/222 promote proliferation by suppressing targets that normally serve to repress proliferative pathways.

miR-221/222 in resistance to apoptotic stimuli

Overexpression of miR-221/222 serves to protect cancer cells against various forms of apoptotic stimuli, including chemotherapeutics, endocrine therapies, radiotherapy and detached growth conditions. MCF7 cells resistant to cisplatin have increased miR-221/222 expression compared to the wild type cells (47). Antagonizing miR-221 in pancreatic cell lines causes increased apoptosis and sensitized the cells to gemcitabine (124). miR-221 and miR-222 are increased in taxol resistant cells, and addition of miR-221 to breast cancer cells results in increased survival in response to paclitaxel treatment (125). One of the

mechanisms through which miR-221/222 repress apoptosis is through direct targeting of pro-apototic genes, such as PUMA (126) and BMF (106).

Her2/neu amplified breast cancers tend to be resistant to endocrine therapy (127,128). miR-221/222 are high in breast cancers that are positive for Her2/neu, compared to Her2/neu negative breast cancers, and overexpression of miR-221/222 causes MCF7 cells to become tamoxifen resistant (129). miR-221/222 directly target $p27^{KIP1}$ (114) and this is one of the mechanisms through which the cells become tamoxifen-resistant. In xenograft tumors that are resistant to tamoxifen, antagonizing miR-222 sensitizes tumors to tamoxifen (130). miR-221/222 directly target TIMP3, a tissue metalloproteinase inhibitor that normally inhibits tamoxifen resistant tumor growth. In breast cancer cells that have become resistant to tamoxifen through increased miR-221/222 expression, TIMP3 is repressed, and there is a resultant increase in the expression of metalloproteases ADAM17 and ADAM 10, as well as increased growth factor signaling (130).

While MCF7 cells treated with tamoxifen have slightly decreased levels of miR-221/222, cells treated with fulvestrant, either alone or in combination with E2, have increased miR-221/222 expression (131), likely because ER represses miR-221/222 (101), so degradation of ER after fulvestrant binding could relieve repression of miR-221/222. Inhibition of miR-221/222 activity causes decreased proliferation. Fulvestrant resistance is explained in part by the downregulation of $p27^{KIP1}$ and $p57^{KIP2}$ (111,112), and ER (100,101). Increased p-catenin contributes to fulvestrant resistance and E2 independent growth (132). Cells overexpressing miR-221/222 have increased nuclear β-catenin, corresponding to increased β-catenin-mediated transcriptional activity. TGF-β1 blocks proliferation in wild type MCF7s, but not the fulvestrant resistant cells (133,134). However, overexpression of miR-221 or miR-222 in wild type cells increases survival in response to TGF-β1, and antagonizing these miRNAs in resistant cells increases sensitivity (131). Therefore, it is possible that miR-221/222 are involved in switching the effect of TGF-β signaling from tumor suppressive to tumor promotional. The genes and phenotypes regulated by miR-221/222 are depicted in Figure 2.

Regulation of miR-221/222

There is a negative feedback loop between miR-221/222 and ERα. miR-221/222 directly bind to and down-regulate ERα, while ERα binds to estrogen response elements in the promoter of miR-221/222 and represses transcription (101). Other transcriptional repressors of miR-221/222 function in a cell-type specific manner. For example, in AML cells, the AML1 protein binds to the promoter of miR-221/222 and represses transcription (135). In melanoma cells, a transcriptional repressor, PLZF (promyelocytic leukemia zinc finger) binds to the promoter of miR-221/222 (136).

FOSL1 (Fra-1) is part of the AP-1 transcription complex and promotes invasiveness and metastatic potential of breast cancers (137–139). FOSL1 binds an AP-1 site upstream of miR-221/222 and promotes transcription (20). Activation of the RAS/RAF/MEK pathway increased expression of miR-221/222 in basal breast cancer cells via FOSL1 (20), and activation of the MAPK pathway also increases miR-221/222 expression [D. El-Ashry, Personal Communication].

Interplay between the miR-200 and miR-221 families

Perhaps the most convincing evidence that these two families play an important role in epithelial plasticity in breast cancer comes from the White lab, in a study where breast cancer cells were forced to undergo EMT by being grown in mammosphere conditions. The resulting cells had decreased miR-200, and increased miR-221/222 (107). Collectively, as described above, these two families clearly exert opposing effects on polarity, migration and invasion, proliferation, apoptosis, and differentiation.

ZEB1/2 transcription factors promote a mesenchymal phenotype by repressing genes involved in polarity. Therefore, ZEB1/2 is detrimental to an epithelial phenotype, and it is essential that these genes remain suppressed in differentiated epithelial cells. While they are most definitely repressed at the promoter level, epithelial cells have evolved an additional layer of protection against their expression, which is miR-200 mediated repression at the post-transcriptional level. Conversely, miR-221/222 promote expression of ZEB2 indirectly through TRPS1, and therefore these miRNAs tend to only be expressed in cells that have undergone EMT (20).

miR-221/222 directly target and repress Dicer, while miR-200c increases Dicer by a yet to be identified mechanism (21). miR-221/222 are more highly expressed in TNBC (21,100). miR-103/107 have also been demonstrated to directly target Dicer (140); however, an inverse correlation between these miRNA and Dicer has not been as well documented as it has for miR-221/222 which are high in tumors in which Dicer levels are low (TNBC). Thus, miR-221/222 may keep Dicer levels low in poorly differentiated breast cancers (21). Since Dicer is required for the maturation of most miRNAs, this may explain why overall miRNA expression is lower in TNBC than luminal. Dicer is often low in cancers that have undergone EMT (53). Dicer is clearly lower in TNBC than adjacent normal breast epithelial cells, while in luminal A breast cancers the difference between tumor and normal is much less dramatic (Figure 3). Interestingly, TAp63 was recently discovered to suppress metastasis by positively regulating Dicer (141). It is possible that miR-200c increases Dicer through its ability to repress ZEB1, which upregulates deltaNp63 (142), a dominant negative inhibitor of TAp63.Consequently, the miR-221 and miR-200 families may control the global miRNA landscape in normal and cancerous cells by dueling for control of Dicer. Much remains to be explored to fully determine how the influence of these miRNA families over Dicer might control motility and metastasis in normal development and cancer.

Conclusions

The role of miRNAs in tumorigenesis and the power they wield with respect to phenotypic control and tumor behavior is just beginning to be understood. In this review we focus on two of the most dysregulated miRNA families in breast cancer, the miR-200 and miR-221 families. The miR-200 family serves to protect the epithelial phenotype, while simultaneously suppressing EMT and tumorigenesis. The miR-200 family protects against migration/invasion, anoikis- and therapeutic resistance, and stem cell-like properties. Conversely, miR-221/222 promote a mesenchymal-like phenotype, and support tumorigenesis. Expression of miR-221/222 inhibits tumor suppressors and genes involved in

apoptosis, cell cycle inhibition, and miRNA processing. Both miRNA families impinge on two important pathways: EMT through ZEB1/2, and miRNA processing through Dicer.

These two miRNA families promote dueling phenotypes, thus they are coordinately regulated during cellular transformations such as EMT and MET (Figure 4). During oncogenic EMT the miR-200 family is strongly down-regulated, while miR-221/222 are highly up-regulated and the reverse is true during MET. This suggests that not only is each miRNA family important for induction of their respective phenotypes, but that the coordinated inverse regulation of these families is required to fully achieve an epithelial or mesenchymal phenotype and associated functional properties. In contrast to their now quite evident role in breast cancer, to date, these miRNA families have not been specifically examined in the normal human breast or mouse mammary gland, although some of their identified targets are clearly relevant in the normal gland.

Abbreviations

EMT	Epithelial to mesenchymal transition
ZEB1/2	Zinc finger E-box binding homeobox 1/2
UTR	Untranslated Region
MET	Mesenchymal to epithelial transition
MDCK	Madin-Darby Canine Kidney
iPSC	Induced pluripotent stem cell
TGF-β	Transforming growth factor beta
PDGF	Platelet derived growth factor
EGFR	Epidermal growth factor receptor
NCI	National Cancer Institute
VEGF	Vascular endothelial growth factor
ER	Estrogen receptor
MMTV	Murine mammary tumor virus

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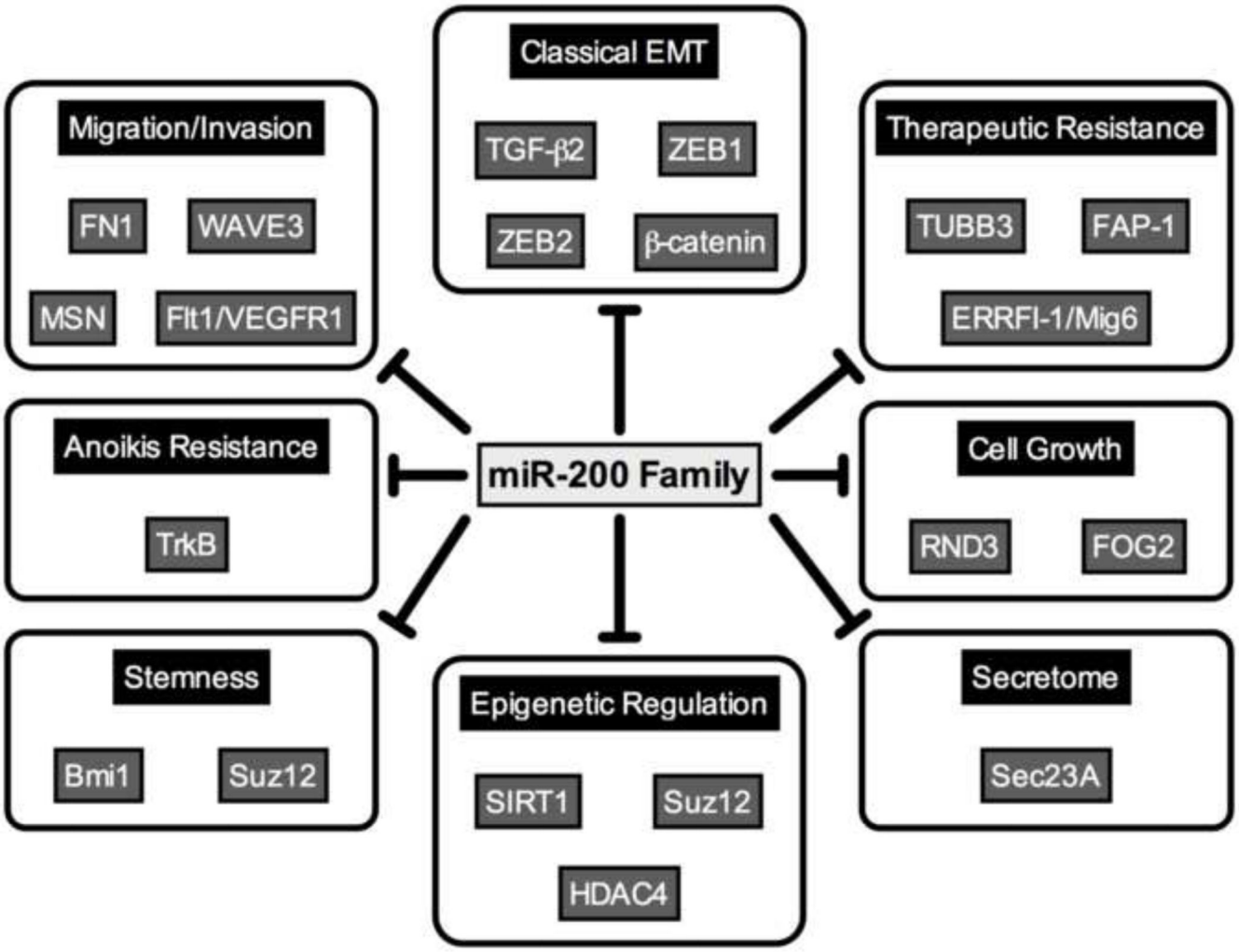

Figure 1.

Direct targets of the miR-200 family. Members of the miR-200 family directly target and down-regulate genes involved in a variety of processes that contribute to tumorigenesis and metastasis. References are included in the text.

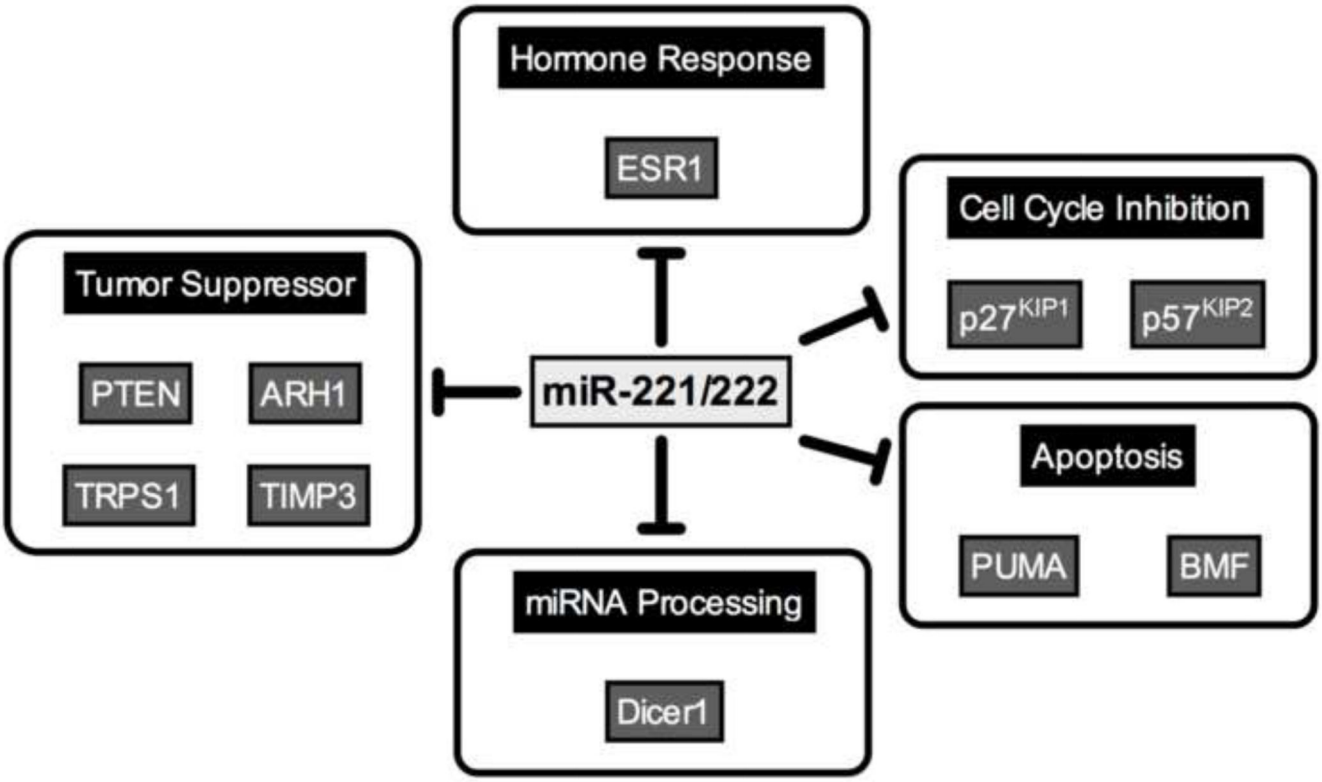

Figure 2.

Direct targets of miR-221/222. miR-221/222 directly target and down-regulate genes associated with differentiation or tumor suppression. References are included in the text.

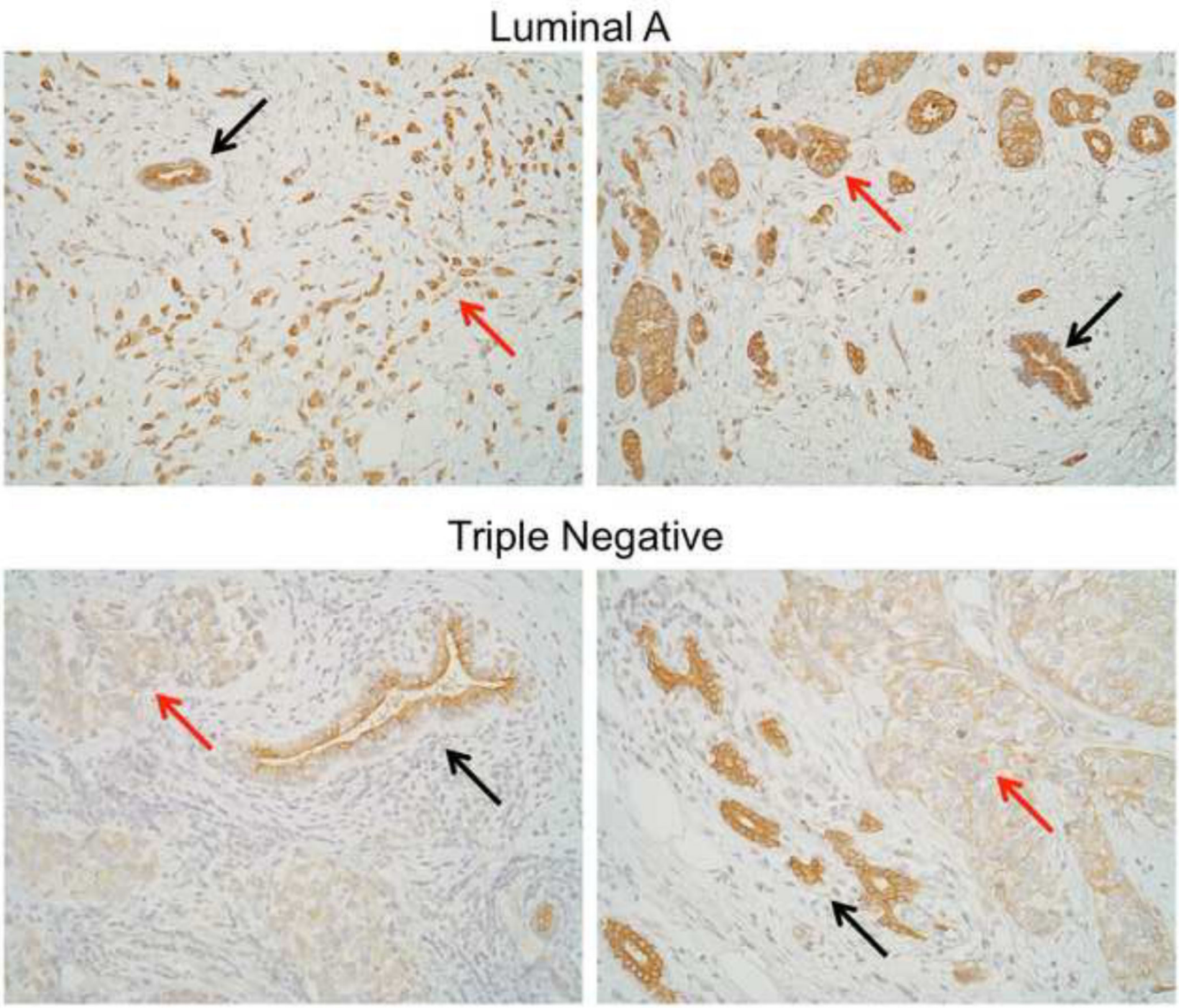

Figure 3.

Dicer protein expression in luminal A and triple negative breast cancer. Formalin-fixed paraffin embedded sections of human breast cancers were stained for Dicer using ab5818 polyclonal antibody (Abcam, Cambridge, MA). Two representative cases each of luminal and triple negative are shown in which adjacent normal glands are present in the same field of vision (top = luminal, bottom = triple negative) with adjacent normal tissue. Red arrows = tumor, black arrows = normal, 200X.

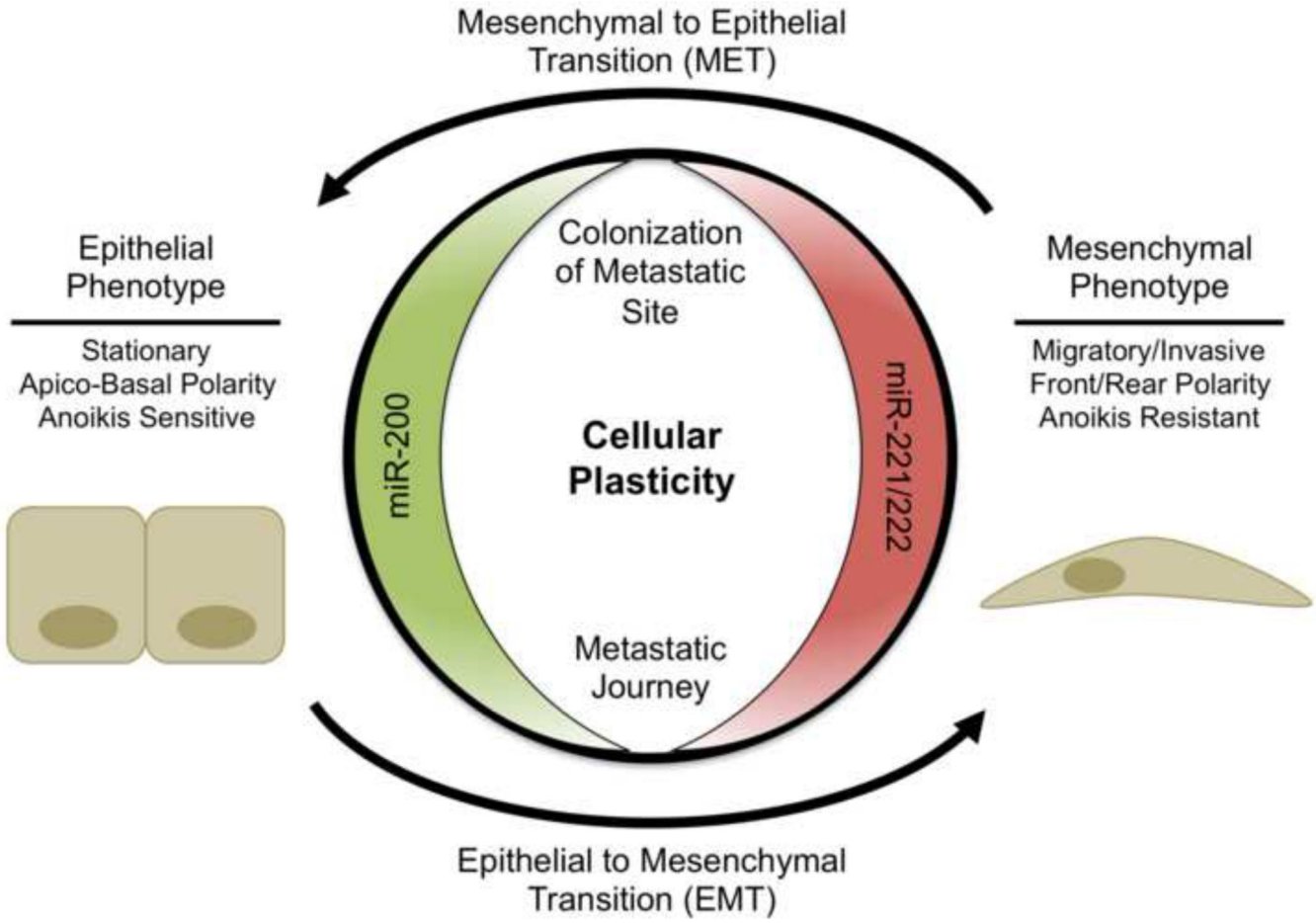

Figure 4.

Phenotypic consequences of miR-200 or miR-221/222 expression. In addition to the roles of miR-200 and miR-221/222 in protecting the epithelial or mesenchymal phenotype, respectively, they are also actively regulated during EMT and MET. Green indicates expression of the miRNA is associated with a less aggressive, epithelial phenotype, while red indicates the miRNA is associated with aggressive behavior.