Progressive and metastatic bladder cancer remain difficult to treat. In this review, we critique seven up-regulated and 25 down-regulated microRNAs in order to identify new therapeutic entities and corresponding targets. These microRNAs were selected with respect to their efficacy in bladder cancer-related preclinical in vivo models. MicroRNAs and related targets interfering with chemoresistance, cell-cycle, signaling, apoptosis, autophagy, transcription factor modulation, epigenetic modification and metabolism are described. In addition, we highlight microRNAs targeting transmembrane receptors and secreted factors. We discuss druggability issues for the identified targets.

In 2020 in the US, 81,400 new cases of bladder cancer (BCa) were diagnosed, with a death toll of 18,000 (1). In the European Union 40,000 cases of BCa are diagnosed annually (2). The vast majority (90%) of cases are urothelial carcinomas which originate in the innermost tissue layer of the bladder and progress to muscle-invasive BCa, finally metastasizing to lymph nodes, bone, lung, liver and peritoneum (3). In this review, we focus on urothelial carcinoma of the bladder as BCa. Non-muscle-invasive BCa has a favorable prognosis and is treated with transurethral resection, intravesical chemotherapy and immunotherapy with Bacillus Calmette-Guerin (3). Only 60% of patients with muscle-invasive BCa are alive 5 years after treatment with neoadjuvant chemotherapy and surgery (4). In patients with metastatic disease, the medium survival time is 15 months (5). In addition to chemotherapy, several new agents have been approved for treatment of BCa such as erdafitinib for fibroblast growth factor receptor 3 (FGFR3) mutations and FGFR-related fusion proteins), enfortumab vedotin (monoclonal antibody to nectin 4 conjugated to monomethyl auristatin E), checkpoint inhibitory monoclonal antibodies such as pembrolizumab and nivolumab directed against programmed cell death protein 1, atezolizumab, durvalumab and atezolizumab directed against programmed death-ligand 1). These agents provide clinical benefit in progressive and metastatic scenarios of BCa but development of resistance is a commonly observed phenomenon (6, 7). Therefore, the identification of new targets and development of new treatment regimens is a very important issue (8-10). In this review, we focus on microRNAs (miRs) with documented activity in preclinical in vivo models as a tool for the identification of new targets and as possible new entities for treatment of BCa.

miRs and their Role in Oncology

miRs are synthesized in the nucleus as precursors, processed and transported to the cytoplasm (11, 12). Finally, one strand of a 22-nucleotide duplex is maintained (guide strand) while the other strand (passenger strand) is degraded (11, 12). Binding of the guide strand to the 3’-untranslated region (3’-UTR) of the corresponding mRNA leads to degradation or translational repression of the target mRNA (11, 12). miRs
can interfere with expression of several target genes and can therefore modulate several pathways and potentially can rewire oncogenic networks (13). miRs can act as tumor suppressors or as oncogenes in a context-dependent manner (14-16). We have recently summarized the role of miRs with respect to the growth and metastasis of breast (17) and prostate cancer (18), non-small cell lung carcinoma (19), and hepatocellular (20) and pancreatic carcinoma (21). The role of miRs in BCa has been reviewed in (22, 23). Here, we focus on BCa-related miRs with activity in preclinical in vivo models as tools for target identification and potential new entities for treatment of BCa.

miRs Up-regulated in BCa

miRs affecting apoptosis, autophagy and signaling
miR-21 targets protein phosphatase 2 regulatory subunit Bα (PPP2R2A). Locked nucleic acid miR-21 blocked anchorage-dependent growth and invasive capacity of RT-112 and 5637 BCa cells in vitro (24) and RT-112 xenografts after tail-vein or intraperitoneal injection (24). PPP2R2A was identified as a target of miR-21 (Figure 1) (24). Suppression of PPP2R2A activates the extracellular signal-regulated kinase (ERK) signaling pathway (24). PPP2R2A functions as a tumor suppressor in BCa and is a determinant of substrate specificity of the heterotrimeric serine/threonine protein phosphatase 2A (25, 26). In non-small cell lung cancer, miR-136 was shown to promote ERK1/2 activation by targeting PPP2R2A (27).

miR-24-3p targets death-effector domain-containing protein (DEED). miR-24-3p (Figure 1) promoted proliferation migration and invasion, inhibited apoptosis and participated in autophagy in T24 and HBC BCa cells (28). miR-24 promoted growth of T24 BCa cells subcutaneously injected into nude mice (28). DEED was identified as a target of miR-24-3p (28). In BCa, miR-24-3p is overexpressed and expression of DEED is low (28). DEED functions as a tumor suppressor and reverses epithelial mesenchymal transition (EMT) by activating autophagy (29, 30). DEED is the final target of a series of events by which cluster of differentiation 95-induced signals are transferred to nucleoli to shut-off cellular biosynthetic activities (31). DEED binds to DNA and reconstitutes mono-nucleosomes and inhibits transcription in a reconstituted in vitro system (31).

miR-135a targets glycogen synthase kinase-3β (GSK-3β). Mao et al. found that miR-135a (Figure 1) was increased in BCa tissue in comparison to adjacent normal tissue. miR-135a increased cell proliferation and migration and reduced apoptosis of T24 BCa cells. They showed in nude mice that miR-135a overexpression stimulated growth of T24 xenografts, whilst its inhibition suppressed xenograft growth. Glycogen synthase kinase-3β (GSK-3β) was identified as a target of miR-135a. miR-135a activated WNT/β-catenin signaling and EMT by inhibition of GSK-3β signaling (32). Other studies have also shown that GSK-3β inhibits WNT signaling, attenuates proliferation and stimulates apoptosis of BCa cells (33, 34). β-Catenin activation is related to BCa progression (35). GSK-3β has been identified as a prognostic marker and potential target in BCa (35).

miR-495 targets phosphatase and tensin homolog deleted on chromosome 10 (PTEN). miR-495 (Figure 1) is overexpressed in BCa and corresponding cell lines. High miR-495 expression was correlated with larger tumor size, advanced TNM stage and lymph node metastasis. miR-495 was shown to promote BCa proliferation and invasion by targeting PTEN (36). In nude mice, miR-495 accelerated the growth of subcutaneous BCa xenografts in association with down-regulation of PTEN (36). PTEN metabolizes phosphatidylinositol 3,4,5 triphosphate, the ligand product of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), thereby opposing activation of the PI3K/AKT serine/threonine kinase 1 (AKT)/mechanistic target of rapamycin (mTOR) signaling network (37-39). In BCa, down-regulation of PTEN has been correlated with poor prognosis, chemoresistance and progression (40). Targeting PI3K/AKT/mTOR is an important pathway for therapeutic intervention in BCa (41).

miR-516A targets PH domain leucine rich containing protein phosphatase 2 (PHLPP2). miR-516A (Figure 1) was shown to be up-regulated in BCa tissues and cell lines. miR-516A mediated proliferation and anchorage-independent growth of UMUC3 and J82 BCa cell lines in vitro and promoted tumor growth in nude mice of UMUC3 and J82 transfected with miR-516A. PHLPP2 has been identified as a target of miR-516A. The miR-516A–PHLPP2 cascade promoted E3 ubiquitin ligase-mediated Beclin1 (BECN1) degradation and thus attenuating autophagy and promoting BCa growth (42). PHLPP2 and its closely related paralog PHLPP1 are members of the protein phosphatase 2C family of Mg2+- and Mn2+-dependent phosphatases (43). They can inactivate signaling of AKT and protein kinase C by dephosphorylation of C-terminal hydrophobic phosphorylation motifs (43, 44). BECN1 is a control protein that assembles cofactors for triggering autophagy induction (45, 46). Autophagy has a multifaceted role in cancer and its function is context-dependent (45, 46).

miRs Affecting Several Targets

miR-193a-3p targets splicing factor serine/arginine rich (SRSF2), plasminogen activator urokinase (PLAU), hypermethylated in cancer 2 (HIC2), lysyl oxidase-like 4 (LOXL4) and transcription factor homeobox C9 (HOXC9)
miR-193a-3p (Figure 2) regulates the activation of chemoresistance-associated signaling pathways. miR-193a-3p was found to have a positive effect on growth and chemoresistance of BCa xenografts after intra-tumoral injection in nude mice. Three direct targets have been identified as mediating these effects: Splicing factor serine/arginine rich (SRSF2), urokinase plasminogen activator (uPA) and hypermethylated in cancer 2 (HIC2) (47). SRSF2 is a regulator of splicing and nuclear-cytoplasmic transportation of mature RNA (48). Interaction of uPA with its receptor protects against anoikis by increasing the level of BCL2 apoptosis regulator-like 1-xL (49), which is in contradiction to the results described above which implicate down-regulation of uPA by miR-193a-3p in protection against apoptosis by a not yet resolved mechanism (47). HIC2 is hypermethylated in cancer and acts as a transcriptional repressor (50). Inhibition of these three targets results in modulation of five signaling pathways: DNA damage, NOTCH, nuclear factor κB (NFκB), MYC and oxidative stress (50).

Another group has identified lysyl oxidase-like 4 (LOXL4) as a direct target of miR-193a, contributing to growth and chemoresistance of BCa cells. miR-193-3p levels were found to be higher in chemoresistant (H-bc and UM-UC-3) compared to chemosensitive BCa cell lines. Intra-tumoral injection of miR-193a-3p promoted growth of 5637 and H-bc BCa xenografts. miR-193a-3p activates the miR-193a/LOXL4/oxidative stress pathway with involvement of transcription factor nuclear facer erythroid 2-related factor (NRF2) (51). LOXL4 is an extracellular copper-dependent amine oxidase which plays a key role in matrix stability and integrity by catalyzing cross-links between collagens and...
elastin, leading to the production of insoluble fibers and is often deregulated in cancer (52). In BCa, LOXL4 has been shown to inhibit tumor growth (53).

Furthermore, it has been shown that miR-193-3p promotes chemoresistance by targeting transcription factor homeobox C9 (HOXC9) (54). HOXC9 participates in the miR-193a/HOXC9/DNA-damage response/oxidative stress pathway axis (54) and is involved in phenotype switching in breast cancer (55).

mir-145 targets forkhead box protein 1 (FOXO1) – stage-dependent function. miR-145 (Figure 2) was shown to be up-regulated in BCa tissues and T24T metastatic BCa cell line. Ectopic expression of miR-145 inhibited anchorage-independent growth of T24 BCa cells, however in T24T BCa cells, promotion of anchorage-independent growth by miR-145 was observed. Up-regulation of FOXO1 and its effector p27 was observed in T24 cells, whilst in T24T cells, these were down-regulated. miR-145 promoted xenograft formation in nude mice injected with T24T transfectants. From a mechanistic point of view, miR-145 was shown to bind to the 3′-UTR of FOXO1 in both T24 and T24T cell lines (56), miR-145 enhanced FOXO1 transcription by inhibiting phosphorylation of signal transducer and activator of transcription 3 (STAT3) at Tyr 705 in T24 cells but not in T24 cells, indicating that these differential effects are mediated by FOXO1. miR-145 was found to inhibit STAT3 phosphorylation at Tyr705 through targeting Janus kinase 2 (JAK2) mRNA coding sequence. JAK2 is responsible for phosphorylation of STAT3. Knock-down of STAT3 impaired miR-145 promotion of xenograft formation by T24T transfectants (56). FOXO1 induces cyclin-dependent kinase (CDK) inhibitor p27 and suppresses cyclin D1 and D2 expression (57). STAT3 is a transcription factor which plays a key role in growth and apoptosis of tumor cells (58, 59). However, according to the data retrieved from the The Cancer Genome Atlas (TCGA) (Figure 3), miR-145 is down-regulated at the RNA steady-state level in BCa.

Down-regulated miRs

miRs affecting cell-cycle-related targets

miR-34b targets cyclin D2 (CCND2) and P2Y purinoreceptor 1 (P2RY1). miR-34b-3p (Figure 4) was shown to repress multi-drug chemoresistance of BCa cells 5637 and EJ. In a nude mouse xenograft model, miR-34b-3p inhibited tumor growth and paclitaxel resistance. CCND2 and P2RY1 have been identified as direct targets of miR-34b-3p (60). CCND2 forms complexes with CDK4/6, which mediate G1/S transition (61). P2RY1 is a G-protein-coupled receptor with ADP as an antagonist, couples to phospholipase C and triggers Ca2+ release from intracellular reservoirs; it is also involved in platelet aggregation (62, 63). The details of how P2RY1 contributes to the phenomena as described above has not yet been resolved.

miR-124 targets CDK4. miR-124 (Figure 4) was found to be down-regulated in BCa tissue samples. CDK4 has been identified as a target of miR-124. miR-124 inhibited growth of HT1197 BCa cells by inducing cell-cycle arrest. miR-124 retarded growth of HT1197 BCa xenografts (64). Cell-cycle deregulation is a key driver of disease progression in BCa and it has been shown independently that inhibition of CDK4/6 controls proliferation of BCa cells (65). CDK4/6 inhibitors are being tested in more than 80 cancer-related clinical trials, including a trial in BCa (9, 66).

miR-124-3p targets aurora kinase A (AURKA). miR-124-3p (Figure 4) was found to be down-regulated in BCa tissues and cell lines: miR-124-3p inhibited proliferation and invasion and induced apoptosis of BCa cells in vitro and inhibited growth of BCa cell xenografts in vivo. AURKA has been identified as a target for miR-124-3p (67). In BCa, AURKA was shown to promote cell-cycle progression, anti-apoptotic signaling, EMT and stem cell-like properties of cancer cells (68). Independent research has indicated that AURKA is a diagnostic biomarker for BCa detection, contributes to its aggressive behavior and predicts poor prognosis (69-71). Orally administrable AURKA inhibitor alisertib is being evaluated in several clinical studies in patients with cancer and is in phase III studies in patients with refractory peripheral T-cell lymphoma (72, 73). In patients with advanced BCa, phase II studies are ongoing (74).

miR-146a-3p targets pituitary tumor transforming gene 1 (PTTG1). miR-146a-3p (Figure 4) suppressed migration, invasion and cell-cycle progression and induced senescence in EJ and T24 BCa cells. miR-146a-3p inhibited growth and metastasis to the lungs of EJ BCa cells after tail vein injection into nude mice. PTTG1 was identified as a direct target of miR-146a-3p. PTTG1 expression was negatively correlated with that of miR-146a-3p in BCa tissues and cell lines (75). PTTG1 overexpression was shown to inhibit p21, an inducer of G1-phase arrest (76). p21 has been shown as a target of PTTG1 in pituitary tumor cells (76). In addition, PTTG1 is involved in cell transformation, aneuploidy and survival (77, 78).

miR-1180-5p targets CDK4/6, cyclin D1 (CCND1) and cyclin D2 (CCND2). miR-1180-5p (Figure 4) attenuated proliferation, inhibited colony formation and induced cell-cycle arrest of T24 and EJ BCa cells. miR-1180-5p suppressed tumorigenicity of EJ cells after subcutaneous transplantation into nude mice. miR-1180-5p induced p21, which down-regulates cell-cycle regulators such as CDK4/6, CCND1 and CCNA2 (80). The activation of the p21 gene by miR-1180-5p is an example of a direct gene activation by a miR, other examples have been reported (79, 80, 81). p21 competitively binds to cyclins and...
reduces the formation of cyclin–CDK complexes (82). CDK4 and CDK6 are among the targets of p21 (83). CDK4/6 inhibitors palbociclib and ribociclib have been approved for treatment of hormone receptor-positive human epidermal growth factor receptor (EGFR) 2-negative metastatic breast cancer (84). Preclinical validation studies show that CDK4/6 inhibitors might be considered as clinical candidates for treatment of BCa (66, 85).

miR-3619-5p targets CDK2 and β-catenin. Expression of
miR-3619-5p (Figure 4) and p21 was found to be reduced in BCa tissues and cell lines, and associated with cancer progression. Low expression of p21 or decrease in both p21 and miR-3619-5p were associated with poor survival in patients with BCa (86). miR-3519-5p inhibited proliferation, migration, invasion, EMT and survival of 5637 and T24 BCa cells. Growth of T24 tumors in nude mice was delayed by overexpression of miR-3619-5p. miR-3619-5p was shown to up-regulate p21 by interaction with its promoter and targets binding sites in the 3'UTRs of CDK2 and β-catenin (86). Low p21 expression predicted tumor recurrence and poor prognosis in BCa (87). β-Catenin-mediated WNT signaling is correlated with lymph node density and its inhibition results in induction of apoptosis and lymphangiogenesis. miR-3619-5p targets EGFR. EGFR has been identified as a direct target of miR-202. Knock-down of EGFR inhibited cell proliferation, invasion and migration in T24, BIU87 and EJ Ba cell lines. (98). EGFR expression is deregulated in BCa and is correlated with disease progression (99, 100). EGFR is involved in the pathogenesis and progression of several types of cancer (101). EGFR is a possible target for treatment of BCa (102).

miRs Targeting Transmembrane Proteins and Secreted Factors

miR-31 and miR-328-3p target integrin α5 (ITN A5). miR-31 (Figure 5) was down-regulated in BCa tissues and its expression correlated with individual progression. In T24 and 5637 BCa cells, miR-31 suppressed proliferation, invasion and migration and increased sensitivity to mitomycin C in vitro. ITN A5 has been identified as a target of miR-31 and inhibited AKT and ERK pathways in BCa cells. In nude mice, T24 BCa cells overexpressing miR-31 exhibited reduced tumor growth and increased sensitivity to mitomycin C (90). It has been shown that ITN A5 facilitates BCa invasion through enhanced contraction forces (91).

miR-328-3p (Figure 5) was down-regulated in BCa tissues and inhibited tumorigenesis by ITN A5 and inactivation of the PI3K pathway in BCa. Expression of miR-328-3p in T24 BCa cells inhibited proliferation in vitro and tumor growth in nude mice (92). Furthermore, a positive correlation between ITN A5 expression and malignant phenotype has been found in BCa (93).

miR-122 targets vascular endothelial growth factor (VEGFC). miR-122 (Figure 5) was down-regulated in BCa. miR-122 was shown to inhibit proliferation, migration, invasion and colony formation, and sensitized HT1376 BCa to cisplatin treatment. In HT1376 xenografts, miR-122 inhibited tumor growth and angiogenesis. VEGFC has been identified as a direct target of miR-122 (94). VEGFC participates in tumor angiogenesis and lymphangiogenesis. It is expressed in endothelial and tumor cells and mediates VEGFR2 and VEGFR3 signaling (95, 96). In BCa, VEGFC expression is correlated with lymph node density and microvessel density (97).

miR-202 targets EGFR. A strong correlation between down-regulation of miR-202 (Figure 5) in BCa tissue and clinical characteristics such as T-classification, N-classification, recurrence and mortality was noted. In vitro, miR-202 inhibited cell proliferation, migration and invasion by T24, BIU87 and EJ BCa cells. Overexpression of miR-202 in these cells inhibited their growth as xenografts in nude mice after subcutaneous implantation. EGFR has been identified as a direct target of miR-202. EGFR is a possible target for treatment of BCa (102).
miR-608 targets flotilin 1 (FLOT1). miR-608 (Figure 6) was found to be down-regulated in BCa. In vitro, miR-608 induced G1 phase arrest and inhibition of colony formation by T24 and UMUC3 BCa cells. UMUC3 cells transfected with miR-608 gave rise to slower growing tumors in nude mice (115). FOXO3a suppressed the expression of CCND1 and other related cell regulators by inducing tumor suppressors p21 and p27 (115-117). Up-regulation of miR-608 led to inhibition of AKT/FOXO3a signaling in BCa cells. FLOT1 has been identified as a direct target of miR-608 (115). FLOT1 is a scaffolding protein of lipid raft microdomains and a highly conserved lipid raft marker (118, 119). It is involved in signal transduction, cell adhesion, cytoskeleton remodeling, endocytosis and acts as a signaling mediator by anchoring various receptors of signaling pathways to the cell membrane (118, 119). The mechanistic underpinnings of inhibition of AKT/FOXO3 signaling and FLOT1 remain to be worked out. Independent research has shown overexpression of FLOT1 is involved in proliferation and recurrence of BCa (120). The tractability of FLOT1 for drug discovery is a critical issue.

miR-4324 targets RAC GTPase activating protein-1 (RACGAP1). miR-4324 (Figure 6) was shown to be down-regulated in BCa tissues. In T24 and UMUC3 BCa cells, miR-4324 inhibited growth, invasion and migration, induced G0/G1 phase cell-cycle arrest and mediated a reduction of colony-forming ability. In nude mice, UMUC3 cells transfected with miR-4324 exhibited reduced tumor growth after subcutaneous implantation and reduced lung metastasis after injection into the tail vein. RACGAP1 was identified as the target of miR-4324. RACGAP1 was shown to mediates doxorubicin resistance in BCa cell line UMUC3. miR-4324 is an estrogen receptor 1 target gene which is down-regulated by promoter methylation (121). RACGAP1 increases expression of STAT3 and mediates its translocation into the nucleus and acts as a nuclear chaperone for STAT3 (121-123). RACGAP1 was also found to regulate the activation of GTPases, RAS-related C3 botulinum toxin substrate 1 (RAC1) and cell division control protein 42 homolog (CDC42) trigger cytokinesis and cytoskeletal reorganization, regulating chemotaxis, cell polarity, migration and metastasis (124). RACGAP1 mediated recurrence and metastasis and was also found to regulate the polycomb repressive complex 1, which promotes cytoskeletal and transcriptional pathways enhancing cell motility (125). Targeting the miR-4324–RACGAP1–STAT3-estrogen receptor 1 feedback loop is a potential target for drug discovery in BCa. Tractability of RACGAP1 for drug discovery is a pending issue.

miRs Affecting Apoptosis

miR-138-5p targets survivin. miR-138-5p (Figure 6) was shown to inhibit proliferation of T24 BCa cells in vitro and growth of T24 transfectants in nude mice after subcutaneous implantation. Survivin has been identified as a direct target of miR-138-5p (126). Survivin is a member of the inhibitor of apoptosis (IAP) family of anti-apoptotic proteins containing a single baculovirus IAP repeat domain (126-
Survivin protects cancer cells from apoptosis and autophagy, localizes to the mitotic spindle and interacts with tubulin during mitosis, activates AKT and PI3K signaling, induces angiogenesis and stemness and is a direct target of the WNT signaling pathway (127, 128). Research has shown that survivin can induce proliferation of BCa cells (129). Expression of survivin is correlated with tumor grade and worse prognosis in patients with BCA (130, 131). Survivin is an important target for anticancer drugs, however, clinical studies did not reach the projected endpoints. Therefore, new optimized compounds need to be developed (132).

**miR-200c targets X-linked inhibitor of apoptosis (XIAP).** miR-200c (Figure 6) expressed in T24 BCa cells attenuated lung metastasis after tail vein injection into nude mice and improved survival. XIAP was identified as a target of miR-200c. Expression of XIAP in BCA tissues was correlated with metastasis (133). XIAP contains a baculovirus IAP repeat, a ubiquitin-binding domain and a carboxy-terminal RING finger which catalyzes the ubiquitylation of caspases 3, 7 and 9 (134). XIAP can be inhibited by small molecules targeting the BIR-domain or mimetics of the natural XIAP antagonist, referred to as second mitochondria-derived activator of caspase mimetics (135, 136). XIAP promoted BCA cell survival and invasion in vitro and lung metastasis in nude mice (137, 138). XIAP has been identified as a prognostic marker of early recurrence of non-muscular invasive BCA (139). ASTX 660, a non-peptidometic small-molecule antagonist of XIAP and cellular inhibitor of apoptosis 1/2 (cIAP1/2) is being clinically evaluated in patients with advanced cancer and lymphoma (140).

**miRs Interfering With Autophagy**

miR-154 targets autophagy-related 7 (ATG7). miR-154 (Figure 6) has been shown to be down-regulated in BCA and to suppress proliferation, migration and invasion of T24 and UMUC3 BCA cells in vitro. In nude mice, miR-154 inhibited proliferation and tumorigenesis of T24 BCA cells. ATG7 was identified as a direct target of miR-154 (141). ATG7 is essential for autophagy and autophagosome formation (142). Autophagy can prolong survival of cancer cells but can also activate tumor-suppressor genes in a context-dependent way (143). In BCA cells, inhibition of autophagy causes apoptosis and attenuation of invasion and migration via EMT inhibition (144, 145). ATG7 is overexpressed in BCA and its inhibition might be a promising strategy for treatment of BCA (146).

**miR Targeting Transcription Factors**

miR-15 targets B-lymphoma Moloney murine insertion region 1 (BMI1). miR-15 (Figure 6) was found to be down-regulated in BCA tissues and its expression level inversely correlated with survival. miR-15 inhibited proliferation, migration and invasion of T24, Blu87 and HT1376 BCA cells. miR-15 inhibited EMT and PI3K/AKT signaling. In nude mice, growth of T24 cells transfected with miR-15 was inhibited after subcutaneous implantation. BMI1 was identified as a target of miR-15 (147). BMI1 is a member of the polycomb group of transcription factors which can act as repressors of transcription (148). BMI1 acts as an oncogene, inhibits PTEN, represses p16 and p19 (INKA/ARF genes) and is involved in self-renewal of cancer stem cells (148). Independently, miR-15 was shown to inhibit proliferation, migration and invasion of BCA cells (149, 150). BMI1 was indicated as a prognostic marker in patients with BCA. Patients with higher BMI1 expression were found to have shorter survival (151).

miR-370 targets transcription factor SOX12 (SOX12). miR-370 (Figure 6) was reduced in BCA and inhibited proliferation, migration and invasion of 5637 BCA cells. In nude mice, tumor growth of 5637 cells transfected with miR-370 was inhibited after their subcutaneous implantation. Transcription factor SOX12 was identified as a direct target of miR-370 (152). SOX12 contains a DNA-binding high-mobility group domain (153). SOX12 has been poorly validated in BCA (154). However, in colorectal cancer, gastric cancer and hepatocellular carcinoma, SOX12 has been shown to promote proliferation and metastasis (154-156).

**miRs Involved in Epigenetic Modification**

miR-124 targets ubiquitin-like containing PH and ring domains, 1 (UHRF1). miR-124 (Figure 7) inhibited proliferation, colony formation, migration, invasion and vascular mimicry of T24 and J82 BCA cells. Intratumoral injection of miR-124 into T24 xenografts induced suppression of tumor growth in nude mice. UHRF1, a RING-finger like type 3 ubiquitin ligase, was identified as a direct target of miR-124 (157). UHRF1 recruits DNA methyltransferase 1 to hemi-methylated DNA, coordinates methylation and histone modifications, mediates silencing of tumor-suppressor genes and is involved in DNA repair (158). Epigenetic repression of regulator of G-protein signaling by UHRF1 has been shown to promote BCA progression (159). Expression levels of UHRF1 was shown to be negatively correlated with clinical outcomes of patients with BCA (160).
been identified as a target of miR-411. MLLT1 is a histone acetylation reader and plays a role in chromatin remodeling, expression of oncogenes such as MYC and homeobox genes via histone acetylation and is a critical component of the super elongation complex (162). The mechanism by which miR-411 induces p21 still has to be resolved. MLLT1 plays a role as an oncogene in leukemia development (162). Small molecules disrupting the YEATS reader pocket of MLLT1 are candidates for treatment of acute myeloid leukemia (162). In BCa, further validation of MLLT1 is necessary with respect to its role as a therapeutic target.

miRs Interfering With Metabolism-related Targets

miR-1-3p targets glutaminase (GLS). miR-1-3p (Figure 7) expression was reduced in BCa and corresponding cell lines. In BCa cell lines T24 and UMUC3, miR-1-3p inhibited proliferation, invasion and migration in vitro. In nude mice, T24 cells transfected with miR-124 exhibited reduced tumor growth after subcutaneous implantation. GLS was identified as a direct target of miR-1-3p (163). Increased glutamine metabolism (glutaminolysis) is a hallmark of cancer and a key metabolic change in cancer cells (164, 165). Small-molecule inhibitors of GLS are currently under preclinical validation (166). Data derived from the TCGA confirm the down-regulation of miR-1 in BCa tissue in comparison to non-transformed bladder tissues (Figure 3).

miR-145 targets MYC/polypyrimidine tract binding protein 1 (PTBP1)/ muscle pyruvate kinase isoenzymes 1/2 (PKM) axis. miR-145 (Figure 7) was shown to be down-regulated in clinical BCa and corresponding cell lines. In BCa cell lines T24 and 253JB-V, miR-145 inhibited growth and induced apoptosis in vitro. Intra-tumoral injection of miR-145 into 253JB-V xenografts exerted an antitumoral effect in nude mice (167). Inhibition of miR-145 induced differential splicing of PKM isoforms PKM1 and PKM2 in favor of PKM2, which induces the Warburg effect, resulting in increased glycolysis regardless of oxygen availability (167, 168). The observed shift in isoforms was shown to be induced by down-regulation of MYC and up-regulation of PTBP1 (167). PTBP1 is part of the heterogenous nuclear ribonucleoprotein family, which are regulators of splicing (169). MYC has been identified as a direct target of miR-145 (170). miR-145 was shown to impair the MYC–PTBP1–PKM axis and also inhibit mitogen-activated protein kinase/ERK and PI3K/AKT signaling (167). Down-regulation of miR-145 in BCa tissues in comparison to
corresponding normal bladder tissues is confirmed by data from TCGA (Figure 3).

miR-153 targets indoleamine 2,3 dioxygenase 1 (IDO1). miR-153 (Figure 7) is down-regulated in BCa tissues and cell lines. In T24 and UMUC3 BCa cells, miR-153 induced apoptosis, reduced colony formation, and inhibited invasion, migration and EMT in vitro. miR-153 inhibited tube formation in human umbilical vein endothelial cells and angiogenesis in the chorioallantoic membrane assay. In nude mice, miR-153 inhibited tumor growth of T24 xenografts transduced with miR-153 after subcutaneous implantation. IDO1 was identified as a direct target of miR-153 (171). IDO1 is a rate-limiting enzyme in tryptophan metabolism (172). IDO1 also induces interleukin 6 (IL6) which interacts with IL6 receptor and induces STAT3 and VEGF, which promotes angiogenesis (171). IDO1 expression predicted poorer survival in patients with early-stage BCa (173). IDO1 also induces immuno-suppression. Several IDO1 inhibitors are under clinical evaluation in patients with cancer (174). Recently, IDO1 inhibitor epacadostat has failed in patients with melanoma (175). Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma did not improve progression-free survival or overall survival. The exploration of the clinical value of IDO1 inhibitors in combination with other agents is ongoing.

miR-612 targets malic enzyme 1 (ME1). miR-612 (Figure 7) was down-regulated in BCa tissues and cell lines. In T24 BCa cell line, miR-612 reduced tumor growth, colony formation, migration, invasion and EMT in vitro and in xenografts in nude mice after subcutaneous implantation. ME1 has been identified as a direct target of miR-612 (176). ME1 links the glycolytic and citric cycles and is important for NADPH production, glutamine metabolism and lipogenesis (177). ME1 is a mediator of EMT by inhibition of expression of E-cadherin and induction of membrane metalloproteinase 9, N-cadherin and vimentin (177). ME1 was shown to promote EMT and metastasis in hepatocellular carcinoma and is a marker for poor prognosis (178).

miRs Affecting Several Targets

miR-582-5p and miR-3p target protein geranylgeranyl transferase type 1 subunit β (PGGT1B), leucine-rich repeat
kinase 2 (LRRK2), DIX domain-containing 1 (DIXDC1) and RAS-related GTP-binding protein 27A (RAB27A). miR-582-5p and miR-3p (Figure 7) were down-regulated in BCa tissues and corresponding cell lines. miR-582-5p and miR-3p interfered with proliferative and invasive abilities of UMUC3, J82 and TCCSUP BCa cells. In an orthotopic BCa xenograft model, tumor growth and lung metastasis of UMUC3 BCa cells were inhibited by transurethrally delivered miR-582-5p or miR-3p complexes. PGGT1B, LRRK2, DIXDC1 and RAB27A were identified as targets of miR-582-5p and miR-3p (179). PGGT1B geranylates ras homolog family members, disrupts F-actin organization and promotes motility, invasion and metastasis (180, 181). LRRK2 directly phosphorylates AKT1, resulting in cell survival and prevention of apoptosis (182). DIXDC1 up-regulates CCND1, down-regulates p21 and inhibits G1/S arrest (183). RAB27A functions as a promoter of proliferation, invasion and metastasis (184). RAB27A was shown to promote BCa cell proliferation and chemoresistance through regulation of NFκB signaling (185).

miR-502 targets CCND1, DNA methyltransferase 3β (DNMT3B) and nucleolar protein 14 (NOP14). miR-502 (Figure 7) has been shown to be frequently down-regulated in BCa. miR-502 inhibited proliferation of T24 and UMUC3 BCa cell lines in vitro and growth of UMUC3 cells transfected with miR-502 in nude mice after subcutaneous implantation (186). miR-502 directly targeted CCND1 (187), NOP14 (188, 189) and DNMT3B (190, 191). CCND1 forms a complex with CDK4 and CDK6, which are responsible for G1/S transition (187).

Conclusion

We have identified seven miRs up-regulated and 25 down-regulated in BCa-related tissues with efficacy in preclinical BCa-related in vivo models. Up-regulated miRs can be inhibited by single-stranded RNAs, with 12-25 nucleotides complementary to the corresponding mRNA (192) or with miR sponges which contain multiple miR-binding sites which compete with the specific mRNA for binding of the corresponding miR (193). Inhibition of miRs can also be achieved with small molecules which interfere with their transcription or their secondary structure, but specificity issues of the identified compounds are a major issue (194, 195). Candidates drug targets are miR-21, miR-24-3p, miR-135a, miR-495, miR-516A and miR-193a-3p (Figures 1 and 2). Inhibition of miR-193a might be of relevance in overcoming chemoresistance to several drugs (Figure 2). Expression of miR-145 is context-dependent and therefore not a priority target. In the case of PPP2R2A, DEED, PTEN, PHLPP2 and FOXO1 (Figures 1 and 2) druggability due to the absence of protein pockets or unresolved interactions with other proteins is a critical issue with the exception of GSK-3β.

Down-regulated miRs can be substituted by reconstitution therapy, their corresponding targets are candidates for inhibition by small molecules or antibody-based entities. Functional reconstitution of miRs can be achieved with miR mimetics, double-stranded RNAs designed to mimic endogenous mature miRs or to express the corresponding miR with plasmid- or virus-based expression vectors in corresponding recipient cells (195, 196). Analysis of down-regulated miRs provides several promising miRs for reconstitution therapy and targets for inhibitory compounds. All of the down-regulated miRs need to be validated in more detail with respect to their propensity for functional reconstitution and resulting physiological consequences. miR-34b, miR-124, miR-146-3p, miR-1180-5p and miR-3619-5p have targets such as CDK2/4/6 and AURKA which are druggable and which have a high priority in development of BCa agents (Figure 4) (9). miR-31, miR-328, miR-122, miR-202 and miR-210-3p have targets such as INTA5, VEGFC, EGFR and FGFR1 (Figure 5), which are candidates for further validation studies. In the signaling category, miR-100 points to mTOR as a target (Figure 5). Validation of mTOR inhibitors is actively being pursued in BCa-related settings (9). miR-608 and miR-4324 have FLOT1 and RACGAP1 as targets but druggability is a critical issue (Figure 6). miR-138-5p and miR-200c target anti-apoptotic proteins such as survivin and XIAP, corresponding inhibitors for which are already under validation in clinical studies (Figure 6). miR-154 identifies autophagy protein ATG7 and transcription factors, and miR-15 and miR-370 have BMI and SOX12 (miR-370) as possible targets for therapeutic intervention in patients with BCa, but druggability is a critical issue (Figure 6). miR-124 and miR-411 identify epigenetic modifiers such as UHRF1 and MLT1 as new targets for small-molecule epigenetic modifiers but here tractability issues also have been resolved (Figure 7). miR1-3p, miR-145a, miR-153 and miR-612 have metabolism-related targets such as GLS, PKM1 and -2, IDO1 and ME1 as possible new targets for intervention with small molecules. Inhibitors of these targets are under preclinical validation or are under clinical investigation as outlined in the previous sections.

Interestingly, miR-145, targeting FOXO1 or PTBP1/PKM, can be up- or down-regulated in BCa-related cell lines and tissues (Figures 2 and 7). miR-124c targets AURKA, CDK4 and UHRF1 (Figures 4 and 7) with in vivo efficacy against each of these targets, miR-502 and miR-582-3p and miR-5p modulate several targets. The contributions of each target to in vivo efficacy have to be resolved in further detail (Figure 7).

Various issues have to addressed in the preclinical validation process, case by case. These issues are not discussed in further detail in this review. Critical issues are: The optimization of pharmacokinetic and pharmacodynamic properties due to removal by the reticulo-endothelial system, renal excretion, entry into tumor cells and efficiency of intracellular endosomal
escape. Further critical issues are hybridization-dependent and -independent side-effects, immunogenicity, hematological and hepatotoxicity, and cytokine-release syndrome (195-202). Recently the field of miR-based therapeutics has witnessed several setbacks, mainly due to toxicity issues (203). The upcoming years will show whether proof-of-concept clinical studies with miR-based therapeutics are feasible.

Conflicts of Interest

FB is and UHW was an employee of Roche.

Authors’ Contributions

FB and UHW jointly conceived and prepared the manuscript.

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