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Review

Long Non-coding RNAs Sponging MicroRNAs With Efficacy in Preclinical *In Vivo* Models of Esophageal Squamous Cell Cancer

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Abstract. *Esophageal cancer is of two subtypes: Esophageal adenocarcinoma and esophageal squamous cell carcinoma (ESCC). Both are associated with a dismal prognosis. Therefore, the identification of new targets and treatment modalities is an issue of paramount importance. In this review, we focus on long non-coding RNAs (lncRNAs) which have been shown to mediate efficacy in preclinical in vivo models of ESCC by sponging microRNAs. Searching the literature, we identified four lncRNAs which were down-regulated and 23 which were up-regulated in comparison to corresponding normal tissues. The down-regulated lncRNAs lead to up-regulation of oncogenic pathways and down-regulation of tumor suppressors. The up-regulated lncRNAs target transcription factors, transmembrane receptors, cell-cycle related proteins, actin-binding proteins, signaling pathways, enzymes including epigenetic modification factors, cellular transport proteins and other categories. We describe reconstitution and inhibition of function of the corresponding lncRNAs and comment on validation and druggability of the identified targets.*

Esophageal cancer is the seventh most common cancer with 570,000 new cases worldwide (1). Two major subtypes have been identified: esophageal squamous cell carcinoma (ESCC), esophageal adeno-carcinoma (EAC). Gastroesophageal cancer at the junction between esophagus and stomach is a special cancer entity (2). ESCC originates from esophageal squamous epithelium, whilst EAC is derived from glandular cells of the esophagus (2). ESCC is the predominant subtype in Southern Asia and Africa, whereas EAC is the most frequent subtype in Europe and Northern America (3). Esophageal cancer is treated by endoscopic resection, surgery, chemotherapy, radiotherapy, and chemo-radiotherapy (4-6). Nevertheless, the overall 5-year survival rate only reaches from 15 to 25% (7). In addition to 5-fluorouracil, capecitabine (Xeloda) and irinotecan, pembrolizumab (anti-programmed death 1) and nivolumab (anti-cytotoxic T-lymphocyte-associated protein 4) have been approved for treatment of esophageal cancer (8, 9). In addition, drugs interfering with the epidermal growth factor (EGFR), vascular endothelial growth factor/receptor (VEGF/VEGFR), hepatocyte growth factor (HGF)/c-MET and NOTCH signaling, histone deacetylase and histone acetyltransferases, DNA methyltransferase, histone modification and immunotherapy-related targets are under preclinical and clinical development for treatment of ESCC (3, 10) indicating that identification of new targets and treatment modalities is a high priority issue for patients with esophageal cancer.

In this review, we describe long non-coding RNAs (lncRNA) which sponge micro-RNAs (miRs) as targets for new treatment modalities of ESCC and tools for identification of new targets of ESCC. We describe lncRNAs with proven efficacy in ESCC-related preclinical *in vivo* models as a single agent.

LncRNA

In mammals non-coding RNAs can be found as housekeeping RNAs such as ribosomal RNA, transfer RNA, small nuclear RNAs and small nucleolar RNAs, as well as regulatory RNAs such as miRs, short interfering RNAs (siRNA) and lncRNAs (11). LncRNAs are defined as transcripts with a length of larger than 200 nucleotides (12). It has been estimated that more than 60,000 lncRNAs exist

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in humans (13). LncRNAs can be derived from intergenic regions, introns, overlapping protein coding regions or are transcribed in the opposite direction to a protein-encoding gene (14). Their functional role for life and brain development has been established by several knockout mouse models (15). Numerous functions have been assigned to lncRNAs such as promoting of transcription, as decoys to repress transcription, recruitment of chromatin modifying enzymes, as scaffolds for proteins, organization of nuclear architecture, translational regulation, modulators of cell signaling pathways and protein stability, as well as regulators of subcellular stability and sponging of miRs [reviewed in (16, 17)]. In cancer, lncRNAs can act as tumor suppressors and oncogenes in a context-dependent manner and they are involved in all stages of pathogenesis including tumor growth and metastasis as well as angiogenesis and tumor-microenvironment interactions (18-20).

In this review, we focus on lncRNAs which sponge miRs as a predominant mode of action and mediate *in vivo* efficacy in ESCC-related preclinical models.

LncRNAs Down-regulated in ESCC

LncRNA Homo sapiens phosphoglucomutase like protein 5 antisense RNA1 (PGM5-AS1) targets phosphatase and tensin homolog (PTEN). PGM5-AS1 (Figure 1) was down-regulated in ESCC and correlated with poor differentiation, tumor, node, metastasis (TNM) stage and lymph node metastasis and may represent a potential biomarker for ESCC (21). It suppressed proliferation, migration, and invasion of KYSE150 and KYSE450 ESCC cells *in vitro* and tumor growth of KYSE150 cells as xenografts *in vivo* in nude mice (21). PGM5-AS1 was found to be activated by p53 and led to up-regulation of PTEN by sponging of *miR-466* (21). The tumor-suppressive function of PTEN is well-documented (22, 23).

LncRNA-Krüppel-like factor 3 antisense RNA 1 (KLF3-AS1) targets Krüppel-like factor 3 (KLF3). KLF3-AS1 (Figure 1) was poorly expressed in patients with ESCC (24). It reduced migration and invasion and induced apoptosis in Eca109 ESCC cell-derived spheres in *vitro* and tumor growth of Eca109 cells *in vivo* (24). KLF3 was up-regulated through sponging of *miR-185*-5p by lncRNA-KLF3 (24). KLF3 is a zinc finger transcription factor which predominantly represses transcription and is highly expressed in the erythroid lineage (25, 26). It is an important regulator of adipogenesis, erythropoiesis and B-cell development (25, 26). KLF3 regulates cancer cell proliferation, apoptosis, metastasis, tumor–microenvironment interactions and cancer stem cells (CSCs) and its functions are highly context-dependent (25, 26).

LncRNA maternally expressed gene 3 (MEG3) targets Dickkopf homolog 2 (DKK2). LncRNA MEG3 (Figure 1) was down-regulated in ESCC tissues and cell lines (27). MEG3 sponged *miR-4261,* resulting in promotion of proliferation, migration, and invasion of KYSE150 ESCC cells *in vitro (*27). MEG3 inhibited growth of KYSE150 tumors and β-catenin signaling *in vivo (*27). MEG3–*miR-4261* axis regulated DKK2 and wingless integration site (WNT)/β-catenin signaling (27). DKKs are a family of four secreted proteins (DKK-1, -2, -3, -4) which inhibit WNT/β-catenin signaling (28, 29). DKK2 is down-regulated in renal and colorectal cancer (30, 31). Clinicopathological significance of WNT/β-catenin signaling in ESSC was demonstrated (32).

LncRNA tumor suppressor candidate 7 (TUSC7) targets differentially expressed in squamous cell carcinoma 1 (DESC1). TUSC7 (Figure 1) was down-regulated in ESCC tissues and corresponding cell lines (33). TUSC7 acts as a sponge of *miR-224* and overexpression of TUSC7 or inhibition of *miR-224* promoted apoptosis and inhibited chemotherapy resistance in EC9706 and KYSE30 ESCCs *in vitro* and *in vivo* (33).

DESC1 has been identified as a target of *miR-224 (*33). DESC1 belongs to the type II transmembrane family of serine proteases which exhibit signaling functions in cancer (34, 35). DESC1 is down-regulated in ESCC tissues and down-regulates EGFR- and AKT serine/threonine kinase 1 (AKT) signaling in ESCC (36, 37).

Up-regulated lncRNAs Acting as Sponges for MicroRNAs

LncRNAs up-regulating transcription factors.

LncRNA FYVE, Rho GEF and PH domain containing antisense RNA (FDG5-AS1) targets specificity protein 1 (SP1). LncRNA FGD5-AS1 (Figure 2) was found to be overexpressed in ESCC tissues and corresponding cell lines (38). It inhibited proliferation, migration, and invasion of TE-1 and Eca109 ESCC cells *in vitro* (38). Knockdown of *FGD5-AS1* in TE-1 cells reduced tumor growth in nude mice (38). FGD5 sponged *miR-383* which targeted SP1 (38). The latter is a zinc finger protein which either stimulates or inhibits gene promoters (39). Up-regulation of SP1 correlates with progression of ESCC (40, 41). Targeting DNA-binding protein SP1 is being pursued by several drug-discovery approaches (42).

LncRNA HOXA transcript at the distal tip (HOTTIP) targets homeobox transcription factor A13 (HOXA13). LncRNA HOTTIP (Figure 2) promoted proliferation and metastasis of ESCC cells *in vitro* and *in vivo* (43). HOTTIP sponged *miR-30b* and led to up-regulation of HOXA13 and transcription factor SNAIL1 (43). HOXA13 promoted proliferation, invasion, epithelial–mesenchymal transition (EMT) and metastasis of gastric cancer cells *via* activation of extracellular kinase 1 (ERK1) (44, 45). HOXA13 promoted

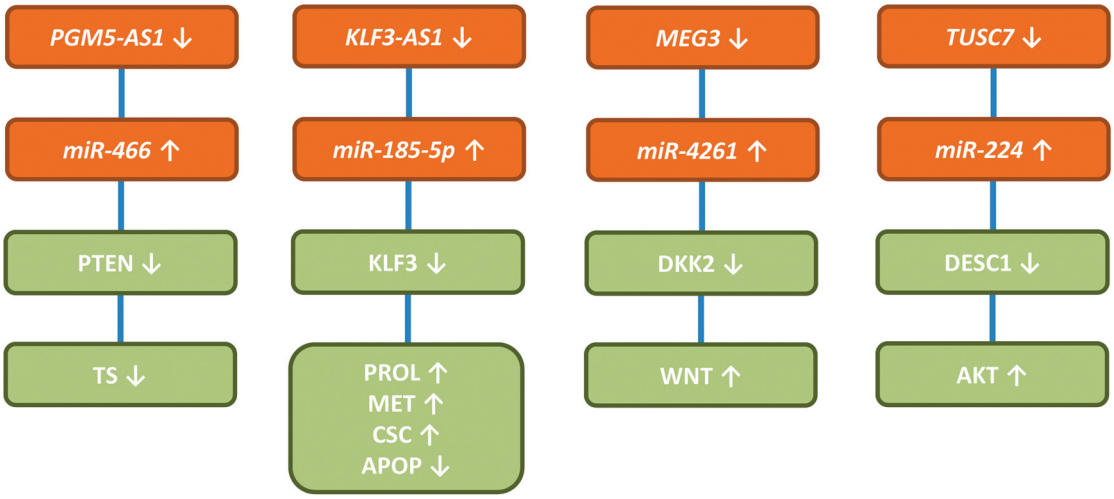

Figure 1. *Down-regulated long non-coding RNAs (lncRNAs) with in vivo efficacy in esophageal cancer related preclinical in vivo models. Up-regulation is indicated by upward arrows, down-regulation by downward arrows. LncRNAs are shown in the first row, sponged microRNAs in the second row, down-regulated targets in the third row and affected signaling pathways and physiological consequences in the fourth row. AKT1: AKT serine/threonine kinase 1; APOP: apoptosis; CSC: cancer stem cell; DESC1: differentially expressed in squamous cell carcinoma; DKK2: Dickkopf-related protein 2; KLF3-AS1: Krüppel-like factor 3-antisense RNA 1; MEG3: maternally expressed gene 3; MET: metastasis; PGM5-AS1: phosphoglucomutase-like-antisense RNA 1; PROL: proliferation; PTEN: phosphatase and tensin homolog; TS: tumor suppressor; TUSC7: tumor suppressor candidate 7; WNT: wingless integration site.*

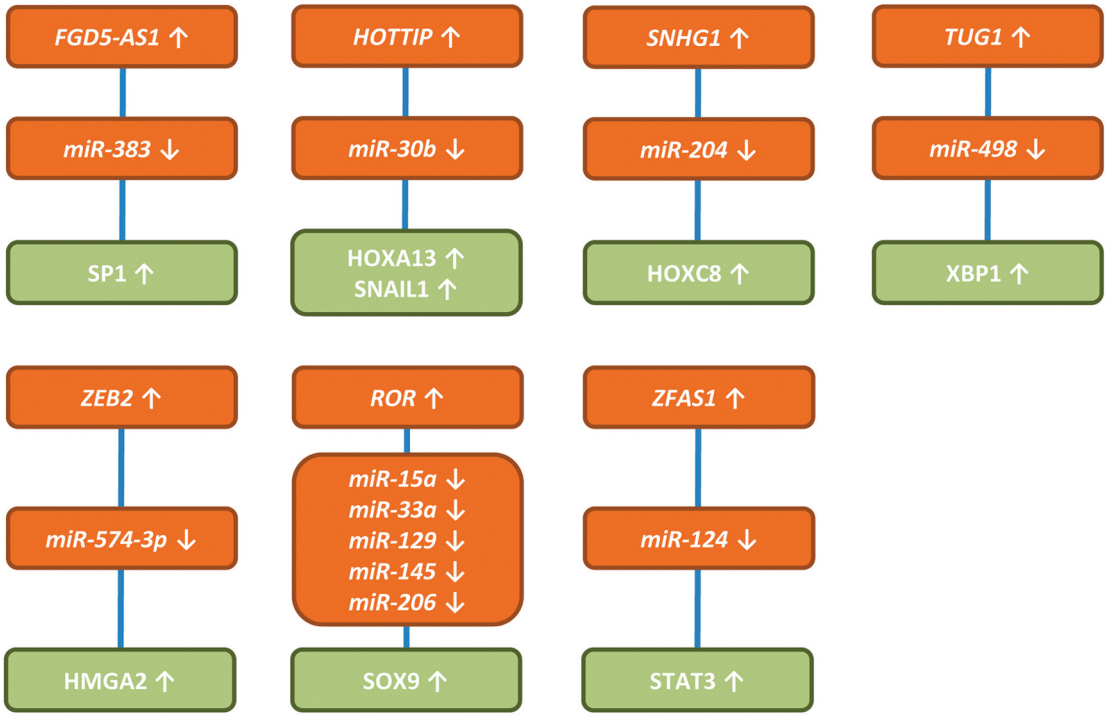

Figure 2. *Up-regulated long non-coding RNAs (lncRNA) with in vivo efficacy in esophageal cancer-related preclinical in vivo models based on up-regulation of transcription factors. Up-regulation is indicated by upward arrows, down-regulation by downward arrows. LncRNAs are shown in the first row, sponged micro RNAs in the second row and down-regulated targets in the third row. FDG5-AS1: FYVE, RhoGEF and PH domain containing 5 antisense RNA1; HMGA2: high motility group protein 2; HOXA13: homeobox protein A13; HOXC8: homeobox protein C8; HOTTIP: HOXA transcript at the distal end; ROR1: RNA regulator of reprogramming; SNHG1: small nucleolar RNA host gene 1; SOX9: SRY-box transcription factor 9; SNAIL1: zinc finger protein SNAIL1; SP1: specificity protein 1; STAT3: signal transducer and activator of transcription 3; TUG1: taurine up-regulated gene 1; XBP1 X-box binding protein 1; ZEB1: zinc finger E-box binding homeobox 1; ZFAS1: zinc finger antisense 1.*

cancer cell growth and predicted poor survival in patients with ESCC (46). Forced expression of HOXA13 conferred oncogenic hallmarks on esophageal keratinocytes (47). The other up-regulated factor, SNAIL1, induces EMT, a process associated with stemness, invasion and tumor progression (48). It has been shown that SNAIL1 confers pro-metastatic functions on ESCC cells (48).

LncRNA small nuclear host gene 1 (SNHG1) targets homeobox transcription factor C8 (HOXC8). LncRNA SNHG1 (Figure 2) was found to be up-regulated in ESCC and its knockdown inhibited proliferation, migration, and invasion of EC9706 and KYSE-150 ESCC cells *in vitro* (49). Silencing of *SNHG1* reduced growth of EC9706 xenografts in nude mice (49). SNHG1 acted as a decoy for *miR-204* which targets HOXC8 (49). The latter exhibits an oncogenic function and is overexpressed in several types of cancer (50). In ESCC, HOXC8 is highly expressed and associated with poor prognosis (51).

LncRNA taurine up-regulated gene 1 (TUG1) targets X box-binding protein 1 (XBP1). LncRNA TUG1 (Figure 2) was up-regulated in ESCC tissues and cell lines (52). *TUG1* knockdown inhibited proliferation, migration, and invasion, but promoted apoptosis of ESCC cells (52). Knockdown of *TUG1* attenuated tumor growth *in vivo* (52). *miR-498* was sponged by TUG1 and XBP1 was identified as a target of *miR-498* (52). XBP1 is a basic region leucine zipper transcription factor and signaling component of the unfolded protein response which promotes proliferation, metastasis, and drug resistance (53, 54). In ESCC, XBP1 promotes proliferation and invasion (55, 56).

LncRNA-zinc finger E-box binding homeobox 2 (LncRNA-ZEB2) targets high mobility group A2 (HMGA2). LncRNA-ZEB2 (Figure 2) was up-regulated in ESCC tissues and cell lines, promoted proliferation, migration and invasion and reduced apoptosis in KYSE150 and KYSE300 ESCC cells (57). Knockdown of lncRNA-ZEB2 inhibited growth of KYSE30 xenografts *in vivo* (57). LncRNA-ZEB2 sponged *miR-574*-3p resulting in promotion of expression of HMGA2 (57) HMGA2 is involved in altering of chromatin structure, apoptosis, cell-cycle progression, DNA repair, senescence, EMT and telomere restoration in cancer cells (58). HMGA2 is overexpressed in ESCC, plays a critical role in tumor progression, and represents a novel diagnostic marker (59).

Intergenic lncRNA regulator of programming (lincROR) targets (sex-determining region Y)-box 9 (SOX9). LincROR (Figure 2) promoted CSC-like properties in EC9706 ESCC cells (60). Intra-tumoral injection of cholesterol-conjugated lincROR siRNA inhibited growth of EC9706 xenografts *in vivo* (60). LincROR sponged miRs-15b, -33a, -129, -145, and -206 which target SOX9 (60). The SOX family comprises more than 20 members that bind to DNA by the high-mobility group domain and are associated with poor prognosis in many types of cancer (61). SOX9 is a transcription factor that regulates many developmental pathways related to stemness, differentiation and generation of progenitors, tumor initiation, proliferation, migration and chemoresistance (61). In breast cancer, SOX9 has been identified as a master regulator of cell fate (62). In ESCC, SOX9 induces stemness and drives phosphoinositide 3-kinase/AKT signaling (63, 64).

LncRNA zinc finger antisense 1 (ZFAS1) targets signal transducer and activator of transcription 3 (STAT3). LncRNA ZFAS1 (Figure 2) was up-regulated in ESCC tissues and Eca109 ESCC cells transmitted ZFAS1 to surrounding cancer cells through exosomes (65). ZFAS1 promoted proliferation, migration, and invasion of ESCC cells by sponging of *miR-124* and up-regulation of STAT3 (65). Overexpression of ZFAS1 promoted tumor growth of Eca109 ESCC cells *in vivo* (65). STAT3 is a transcription factor that regulates proliferation, differentiation, angiogenesis, apoptosis, inflammation, and immune response against tumor cells (66, 67). In ESCC, STAT3 has been shown to inhibit apoptosis and to promote EMT and metastasis (68-70).

LncRNAs Up-regulating Transmembrane Receptors

LncRNA B-RAF activated non-coding RNA (BANCR) targets insulin-like growth factor receptor-1 (IGF1-R). High expression of BANCR (Figure 3) correlated with poor survival in patients with ESCC (71). In KYSE450, KYSE 510 and HET-1A ESCC cells, knockdown of *BANCR* inhibited proliferation, migration, invasion and EMT (71). In KYSE450 cells, knockdown of *BANCR* inhibited tumor growth in nude mice (71). BANCR sponged *miR-338*-3p which targets IGF-1R (71). BANCR regulated the rapidly associated fibrosarcoma (RAF)/mitogen-activated protein kinase (MEK)/ERK pathway in ESCC cells (71). Therapeutic targeting of IGF-1R is being pursued for many types of cancer and small molecule or monoclonal antibody-related entities are undergoing clinical trials (72, 73).

It has been shown that BANCR regulates cell invasion and migration in ESCC through WNT/β-catenin signaling and that up-regulation of *BANCR* correlates with progression and poor prognosis in ESCC (74, 75). Overexpression of IGF-1R is correlated with lymph node metastasis, differentiation, and clinical stage in ESCC patients and its down-regulation in EC9706 cells inhibits proliferation *in vitro* (76).

LncRNA MIRNA 31 host gene (MIR31HG) (miR-34a) and lnc taurine up-regulated gene 1 (lncTUG1) (miR-144-3p) target transmembrane tyrosine kinase c-MET. MIR31HG (Figure 3) regulated the cell cycle and inhibited apoptosis in KYSE30 ESCC cells and its knockdown suppressed KYSE

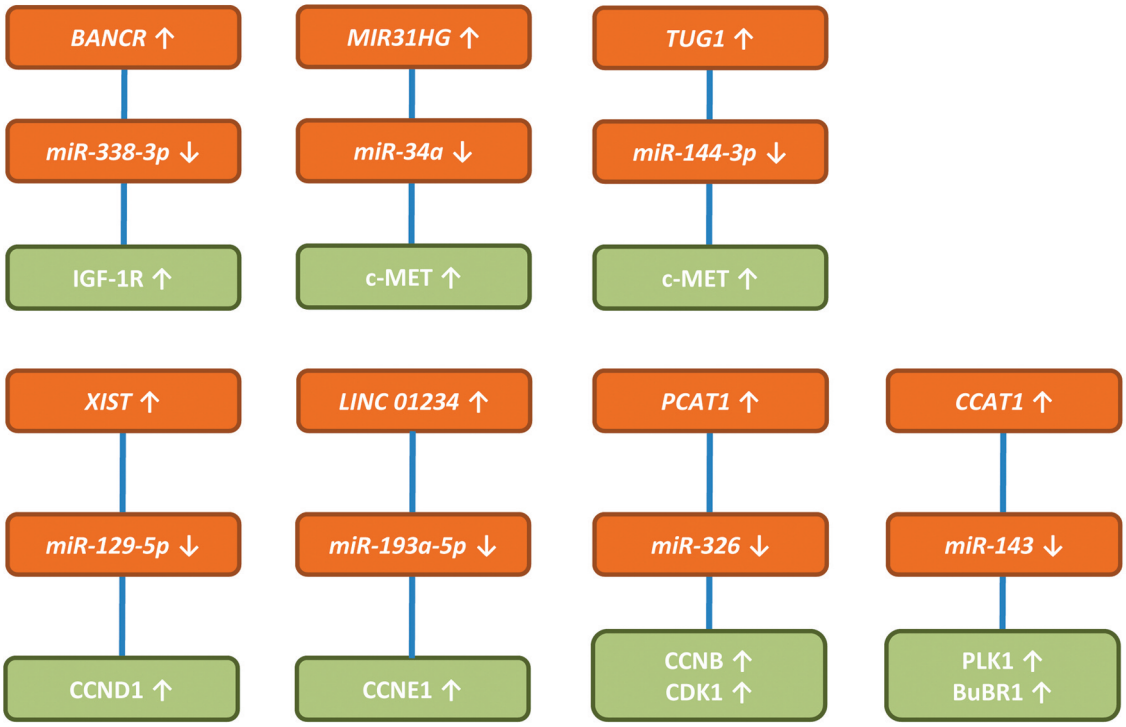

Figure 3. *Up-regulated long non-coding RNAs (lncRNAs) with in vivo efficacy in esophageal cancer-related preclinical in vivo models based on up-regulation of transmembrane receptors and cell-cycle-related targets. Up-regulation is indicated by upward arrows, down-regulation by downward arrows. LncRNAs are shown in the first row, sponged microRNAs in the second row and down-regulated targets in the third row. BANCR: BRAF-activated non-coding RNA; BubR1: Bub1 mitotic checkpoint serine/threonine kinase B; CCAT1: colon-cancer associated transcript 1; CCNB: cyclin B; CCND1: cyclin D1; CCNE1: cyclin E1; CDK1: cyclin-dependent kinase 1; c-MET: tyrosine kinase c-MET; IGF-1R: insulin-like growth factor receptor-1; LINC01234: long non-coding RNA 01234; MIR31HG: miR31 host gene; PCAT1: prostate cancer-associated transcript 1; PLK1: polo-kinase 1; TUG1: taurine up-regulated gene1; XIST: X-inactive-specific transcript.*

30 ESCC cell proliferation and growth in nude mice (77). MIR31HG sponged *miR-34a*, which targets transmembrane tyrosine kinase receptor c-MET (77).

TUG1 (Figure 3) was up-regulated in ESCC tissues and cell lines (78). Its knockdown inhibited proliferation, migration, and invasion *in vitro* in EC9706 and KYSE30 ESCC cells and enhanced radiosensitivity (78). *In vivo*, inhibition of TUG1 retarded tumor growth of KYSE30 cells and increased radiosensitivity in nude mice (78). TUG1 sponged *miR-144*-3p leading to up-regulation of c-MET (78). c-MET is up-regulated in many types of cancers, drives multiple pathways and a subset of cancers are MET-addicted and many small molecule- and monoclonal antibody- based antagonists are undergoing clinical trials in cancer patients (79-81). c-MET is expressed in 43% of ESCC and is an independent prognostic factor in this type of cancer (82-84).

LncRNAs Up-regulating Cell-cycle-related Targets

LncRNA X-inactive-specific transcript (XIST) targets cyclin D1 (CCND1). LncRNA XIST (Figure 3) was up-regulated in ESCC (85). *XIST* silencing repressed cell-cycle progression, migration and invasion and promoted apoptosis in ESCC cells (85). XIST silencing inhibited tumor growth *in vivo* (85). XIST directly interacted with *miR-129*-5p which targets CCND1. Overexpression of CCND1 results in deregulated CDK activity and neoplastic growth by bypassing molecular checkpoints (86). It has been shown that lncRNA XIST promotes development of ESCC by regulation of CDK6 and enhancer of zeste 2 (EZH2) expression (87, 88). Interestingly, *CCND1* G870A polymorphism contributes to the risk of ESCC (89).

LincRNA Linc 01234 targets cyclin E1 (CCNE1). Linc RNA 01234 (Figure 3) was up-regulated in ESCC tissues and by sponging *miR-193a*-5p, cyclin E1 (*CCNE1*) was induced (90). Down-regulation of linc 01234 led to decrease of CCNE1 and BCL2 apoptosis regulator, up-regulation of caspase 3 and p21 in ESCC cells *in vitro* and reduced tumor growth of ESCC cells *in vivo* in nude mice (90). CCNE1 promotes initiation of DNA replication by inducing expression of S-phase-specific genes and centrosome

duplication in late G_1 phase of the cell-cyle (91, 92). Cyclin-dependent kinase 2 (CDK2)–CCNE1 complexes were shown to regulate G_1/S-phase transition (93). It has been shown that CCNE1 is dysregulated in ESCC (94).

LncRNA prostate cancer-associated transcript 1 (PCAT1) targets cyclin B (CCNB) and cyclin-dependent kinase 1 (CDK1). LncRNA PCAT1 (Figure 3) was highly expressed in ESCC specimen and cell lines (95). Knockdown of *PCAT1* in KYSE30 ESCCs decreased soft-agar colony formation, whereas expression of *PCAT1* in KYSE150 and KYSE450 ESCCs increased colony formation (95). Knockdown of *PCAT1* in KYSE 30 cells resulted in smaller tumors in nude mice, whereas expression of PCAT1 in KYSR450 cells gave rise to increased tumor growth in nude mice (95). PCAT1 acted as a sponge for *miR-326* resulting in increased expression of CCNB and CDK1. CCNB is required for S-, G_2- and M-phase progression of the cell cycle (96). CDK1–CCNA and CDK1–CCNB complexes are necessary for S-, G_2- and M-phase progression of the cell cycle (97).

Overexpression of CCNB in ESCC cells induces invasive growth and metastasis and indicates a poor prognosis in patients with ESCC (98).

LncRNA colon cancer-associated transcript 1 (CCAT1) targets polo kinase 1 (PLK1). LncRNA CCAT1 (Figure 3) was shown to be involved in cell proliferation and chemo-resistance of ESCCs (99). *CCAT1* knockdown suppressed tumor growth and enhanced sensitivity to cisplatin in nude mice (99). LncCCAT1 sponged *miR-143,* which regulates expression of PLK1 and Bub1 mitotic checkpoint serine/threonine kinase B (BubR1) (99). PLK1 plays a role in initiation, maintenance, and completion of mitosis, and promotes transformation and tumor progression. PLK1 inhibitors are under clinical investigation in several types of cancer (100, 101). Most promising activity of the PLK1 inhibitor volasertib was seen in patients with acute myeloid leukemia (102). Silencing of *PLK1* causes inhibition of growth and induction of apoptosis in human ESCCs (103). The other target identified, BubR1 is involved in spindle checkpoint function and chromosome segregation (104, 105). In ESCC cells, BubR1 induces resistance to anti-microtubule drugs (106).

Up-regulated lncRNAs Affecting Cell Shape and Actin Binding

LncRNA activated by transforming growth factor 2 (ATB) targets kindlin 2. LncRNA ATB (Figure 4) was up-regulated in ESCC tissues and predicted an unfavorable prognosis in patients with ESCC (107). Knockdown of lncRNA ATB inhibited proliferation and induced cell-cycle arrest in KYSE30 and Eca109 ESCC cells (107). Knockdown of lncRNA ATB suppressed migration, growth, and metastasis of Eca109 xenografts in nude mice (107). LncRNA ATB sponged *miR-200b* and up-regulated kindlin 2. The latter belongs to the 4.1-ezrin-ridixin-moesin (FERM) domain family and interacts with the cytoplasmic tails of β-integrin subunits. This interaction mediates proliferation, migration and invasion of tumor cells, CSC maintenance *via* transforming growth factor β, WNT/β-catenin, p53 and hedgehog pathways (108, 109). In ESCC, it has been shown that *miR-200b* promotes invasion by activating the kindlin 2/integrin β1/AKT pathway (110). *miR-200b* also suppresses invasiveness and modulates the cytoskeletal and adhesive machinery in ESCC cells (111). Kindlin 2 is associated with poor outcome in patients with ESCC (112).

LncRNA plasmacytoma variant translocation 1 (PVT1) targets LIM and SH3 domain protein 1 (LASP1). LncRNA PVT1 (Figure 4) predicted adverse prognosis in patients with ESCC (113). PVT1 promoted proliferation and migration of Eca109 and KYSE150 ESCC cells *in vitro* (113). Knockdown of *PVT1* by small hairpin RNA inhibited growth of Eca109 xenografts in nude mice (113). PVT1 sponged *miR-203* and led to up-regulation of LASP1. The latter is an actin-binding protein which is involved in actin assembly, focal contacts, and focal adhesion formation (114-116). It has been shown that PVT1 promotes viability, invasion, migration and EMT of ESCC cells (117).

LncRNA PVT1 also targets FASCIN 1 (FSCN1). LncRNA PVT1 (Figure 4) also sponged *miR-145* which targets FSCN1 (118). Down-regulation of *PVT1* inhibited viability and promoted apoptosis and G_1 arrest of KYSE-30, KYSE-70, Eca109 and TE-1 ESCC cells *in vitro* and inhibited growth of ESCC cells *in vivo (*118). FSCN1 was identified as an actin-bundling protein implicated in cancer metastasis and recurrence (119). FSCN1 has been shown to promote expression of cluster of differentiation 147 (CD147), VEGFR2 and metastasis-associated protein 1 (MTA1), which are correlated with ESCC progression (120, 121). It has been demonstrated that tumor suppressor *miR-145* can reduce cancer cell migration through regulation of FSCN1 (122, 123).

Up-regulated lncRNAs Targeting Enzymes, Signaling and Epigenetic Modification

LncRNA 440173 (LOC440173) targets histone deactylase 9 (HDAC9). LOC440173 (Figure 4) expression was significantly enhanced in ESCC tissues and corresponding cell lines, and expression correlated with tumor invasion depth, lymph node metastasis and TNM stage (124). *In vitro*, LOC440173 promoted proliferation, migration, invasion and EMT of ESCC cells and *in vivo*, it promoted tumor growth of ESCC xenografts in nude mice (124). LOC440173 sponged *miR-30d*-5p leading to up-regulation of HDAC9

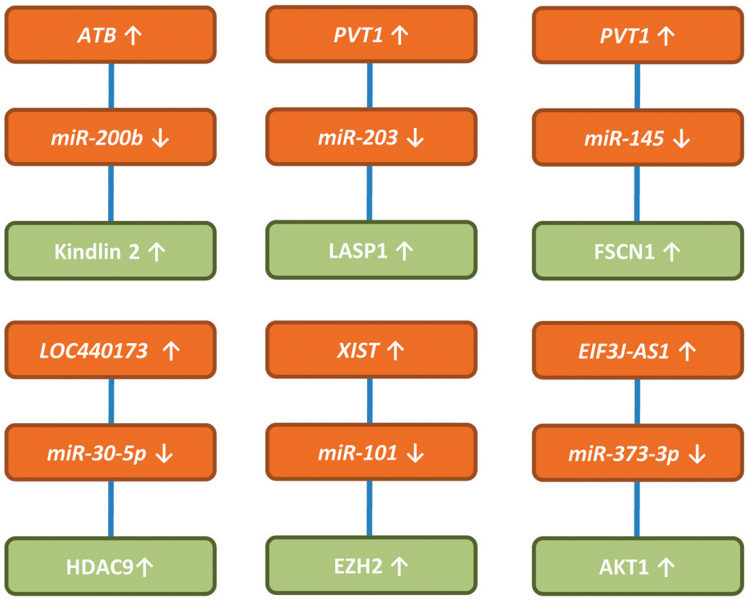

Figure 4. *Up-regulated long non-coding RNAs (lncRNA) with in vivo efficacy in esophageal cancer-related preclinical in vivo models based on up-regulation of components involved in actin binding, cell shape, enzymes, enzymatic activities, and epigenetic modification. Up-regulation is indicated by upward arrows, down-regulation by downward arrows. LncRNAs are shown in the first row, sponged microRNAs in the second row and down-regulated targets in the third row. AKT1: AKT serine-threonine kinase 1; ATB: activated by transforming growth factor β; EIF3J-AS1: eukaryotic translation initiation factor 3, subunit 7 antisense RNA1; EZH2: enhancer of zeste homolog 2; FSCN1: fascin actin-bundling protein; HDAC9: histone deacetylase 9; LASP1: LIM and SH3 domain protein 1; Linc00473: long non-coding RNA Linc00473; LOC440173: lnc RNA LOC440173; PVT1: plasmacytoma variant translocation 1; XIST: X-inactive specific transcript.*

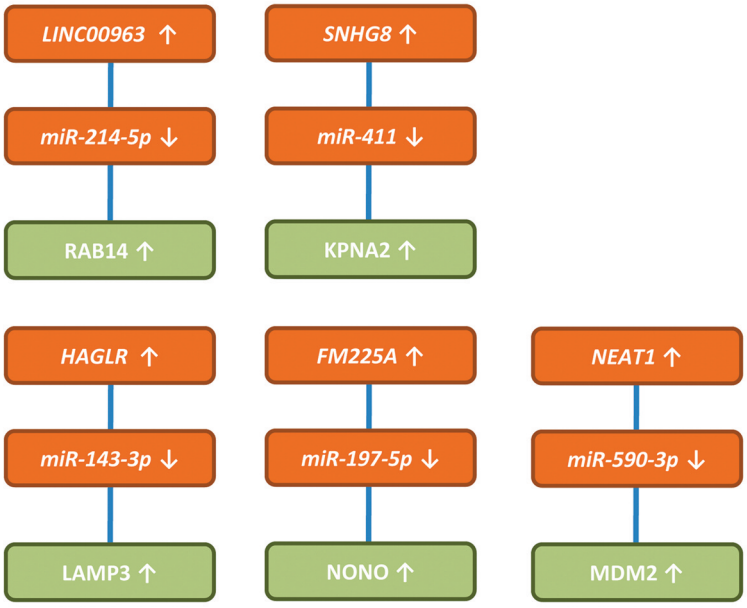

Figure 5. *Up-regulated long non-coding RNAs (lncRNAs) with in vivo efficacy in esophageal cancer-related preclinical in vivo models based on up-regulation of components involved in transport and covering additional categories of targets. Up-regulation is indicated by upward arrows, down-regulation by downward arrows. LncRNAs are shown in the first row, sponged microRNAs in the second row and down-regulated targets in the third row. FAM225A: LncRNA FAM225A; HAGLR: HOXD antisense growth-associated lncRNA; KPNA2: karyopherin α2; MDM2: mouse double minute 2 homolog; LAMP3: lysosome-associated membrane glycoprotein 3; LINC00963: lncRNA LINC00963; NEAT1: nuclear enriched abundant transcript 1; NONO: non-POU domain-containing octamer-binding protein; RAB14: ras-related protein RAB14; SNHG8: small nuclear RNA host gene 8.*

(124). By removing acetyl groups, HDACs reverse chromatin acetylation and alter transcription of oncogenes and tumor-suppressor genes. HDAC inhibitors are being evaluated in clinical studies in many types of cancer [reviewed in (125)]. Overexpression of HDAC9 is correlated with poor prognosis of breast and gastric cancer, hepatocellular carcinoma and pancreatic adenocarcinoma and may be a target for therapeutic intervention for these types of tumors (126-129).

LncRNA XIST targets EZH2. LncRNA XIST (Figure 4) was up-regulated in patients with ESCC and predicted poor prognosis (88). Knockdown of *XIST* inhibited proliferation, migration, and invasion of KYSE30 and KYSE150 ESCCs cells *in vitro* and tumor growth of KYSE150 in nude mice *in vivo* (88). XIST sponged *miR-101* leading to up-regulation of EZH2. The latter is a histone methyltransferase subunit of the polycomb repressor complex (130, 131). EZH2 functions as a master regulator of transcription, is highly expressed in several types of cancer and is mutated in some types of tumors (130, 131). It was shown that *miR-101* suppresses proliferation and migration and induces apoptosis by targeting EZH2 in ESCC cells (132). Overexpression of EZH2 in ESCC was correlated with poor prognosis in patients with ESCC (133).

LncRNA eukaryotic translation initiation factor 3 subunit J-antisense 1 (EIF3J-AS1) targets AKT1. LncRNA EIF3J-AS1 (Figure 4) was increased in tissues of patients with ESCC and correlated with clinicopathological features and poor survival (134). Knockdown of *EIF3J-AS1* in TE-1 and TE-8 ESCC cells impaired proliferation, migration, and invasion *in vitro* and reduced lung metastasis after tail vein injection in nude mice (134). It was shown that EIF3J sponged *miR-373*-3p, resulting in up-regulation of AKT1 (134). The latter is involved in PI3K/mechanistic target of rapamycin signaling and consists of three paralogs AKT1, -2 and -3 (135, 136). The AKT1/mechanistic target of rapamycin kinase pathway is activated in ESCC and activated AKT1 correlates with poor prognosis in ESCC (137, 138).

Up-regulated lncRNAs Involved in Cellular Transport

Long non-coding RNA LINC00963 targets RAB family 14 (RAB 14). LncRNA LINC00963 (Figure 5) was found to be up-regulated in ESCC in comparison to adjacent non-tumor tissues and high expression correlated with poor overall survival (139). Down-regulation of *LIN00963* reduced proliferation of KYSE150 and TE-1 ESCC cell *in vitro* and reduced tumor growth and weight *in vivo* in nude mice (139). It sponged *miR-214*-5p leading to up-regulation of RAB14, a member of the RAS oncogene family (139). RAB14 is involved in intracellular membrane trafficking (140). RAB14 activates mitogen-activated protein kinase signaling to promote bladder carcinogenesis (141) and proliferation of non-small cell lung carcinoma and invasion through YES-associated protein signaling (142).

LncRNA small nucleolar RNA host gene 8 (SNHG8) targets karyopherin α2 (KPNA2). LncRNA SNHG8 (Figure 5) was highly expressed in ESCC tissues and correlated with poor survival (143). Silencing of *SNHG8* inhibited proliferation, migration and invasion and promoted apoptosis of Eca109 and TE-1 ESCC cells *in vitro* and exerted tumor-suppressive and anti-metastatic effects *in vivo* in nude mice (143). LncRNA SNHG8 acted as a sponge for *miR-411,* which inhibited importin subunit KPNA2 (143). KPNA2 is a member of the importin family with key functions in nucleocytoplasmic transport (144). KPNA2 is overexpressed in several types of cancer and is correlated with poor prognosis (145, 146). KPNA2 inhibitor selinexor has been approved for treatment of multiple myeloma and large B-cell lymphoma (147, 148). In ESCC, KPNA2 induces cell proliferation, invasion and is associated with poor differentiation (149, 150).

LncRNAs Targeting Additional Categories of Proteins or RNA

LncRNA homeobox D gene cluster antisense growth-associated long non-coding RNA (HAGLR) targets lysosome-associated membrane protein 3 (LAMP3). HAGLR (Figure 5) was shown to be highly expressed in ESCC and sponged *miR-143*-3p, which suppressed=LAMP3 (151). *In vitro*, down-regulation of HAGLR or up-regulation of *miR-143*-3p inhibited proliferation, migration, and invasion and EMT in EC9706 and Eca109 ESCC cells and growth of ESCC xenografts in nude mice (151). The target of *miR-143*-3p, LAMP3, is a member of the family of LAMP proteins which are located in the membrane of lysosomes (152). Lysosomes can change composition as well as localization during transformation and can release enzymes which promote transformation and metastasis (152). LAMPs are involved in autophagy, phagocytosis, lipid transport and can support tumor growth and metastasis (153). LAMP3 has been identified as a novel biomarker for ESCC and is correlated with poor prognosis (154).

LncRNA family with sequence similarity 225 member A (FAM225A) targets DNA and RNA binding protein non-POU domain-containing octamer-binding protein (NONO). FM225A (Figure 5) was found to be highly expressed in ESCC tissues and correlated with poor prognosis (155). Knockdown of *FM225A* in KYSE30 and KYSE510 ESCC cells suppressed cell proliferation, migration, and invasion *in vitro* (155). Knockdown of *FM225* inhibited tumor growth

in vivo in nude mice (156). FM225A sponged *miR-197*-5p, which negatively regulated NONO (156). The latter is involved in mRNA splicing, DNA unwinding, proliferation, apoptosis, migration, and DNA-damage repair (157-159). Down-regulation of *NONO* induces apoptosis and suppresses growth and invasion of ESCC cells (159).

LncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) targets mouse double minute homolog 2 (MDM2). LncRNA NEAT1 (Figure 5) was highly expressed in ESCC tissues and correlated with lymph node metastasis and distant metastasis (160). NEAT1 promoted proliferation, invasion, migration, and angiogenesis in Eca109 and TE13 ESCC cells and human umbilical vein endothelial cells *in vitro* (161). Knockdown of *NEAT1* inhibited growth of Eca 109 xenografts *in vivo* in nude mice (160). NEAT1 sponged *miR-590*-3p resulting in up-regulation of MDM2 (160). *MDM2* is a proto-oncogene which promotes tumor formation by targeting p53 for degradation and is amplified in several types of tumors (161, 162). Several MDM2 inhibitors have entered clinical trials in patients with cancer (163). The *MDM2* gene is amplified in ESCC, and copy number increase correlates with poor prognosis (164, 165).

Technical Issues Regarding Therapeutic Intervention

We have identified down-regulated and up-regulated lncRNAs deregulated in ESCC tissue specimens, in comparison to corresponding normal tissues, which are able to sponge miRs and mediate efficacy in preclinical *in vivo* models.

Underexpressed lncRNAs are candidates for substitution therapy by forced expression of the corresponding lncRNAs in tumors cells with plasmid- or viral-based expression vectors (18, 166). However, delivery of these vectors to esophageal tumor cells is one of the main issues to be tackled. The identified targets need to be up-regulated with small molecules. However, this approach is seriously limited by specificity issues and therefore has to be ranked as a low-priority approach.

The vast majority of identified lncRNAs sponging miRs are up-regulated in ESSC tissues in comparison to corresponding normal tissues. They are candidates for inhibition with antisense oligonucleotides (ASO) or siRNAs (167, 168). Several RNA-targeted oligonucleotides have received regulatory approval for rare diseases, none is approved yet for cancer (169). Basically, ASO form DNA–RNA structures with a target triggering RNAse H-mediated degradation (170). siRNAs are short 20-24 bps double-stranded RNAs with phosphorylated -5´ ends and hydroxylated -3´ ends with two overhanging nucleotides (170). siRNA-mediated RNA–RNA duplexes are degraded by a pathway dependent on endoribonuclease DICER and Argonaute family members and the corresponding siRNAs can be introduced into cells by transfection (170). shRNAs are artificial RNAs with a tight hairpin loop mediating RNA silencing after transfection with expression vectors into cancer cells (171). ASO-based therapies have witnessed several technological improvements based on medicinal chemistry such as introduction of phosphorothioate linkages, -2´ sugar modifications and conjugation of ligands such as *N*-acetylgalactosamine for delivery to the liver (172). Locked nucleic acids and gapmers are further improvements of stability and function of ASOs (173, 174). Despite these encouraging improvements, several aspects are open to further improvement, especially issues such as delivery, toxicity, and efficacy (175-178). A detailed discussion of these topics is not in the focus of this review.

Conclusion

As shown in Figure 1, we identified four lncRNAs which are down-regulated in ESSC tissues in comparison to corresponding normal tissues. They cover targets PTEN, KLF3, DKK2 and DESC1. The corresponding lncRNAs are potential candidates for replacement therapy.

The lncRNAs up-regulated in ESCC tissues mediate up-regulation of transcription factors (Figure 2), transmembrane receptors and cell-cycle related targets (Figure 3), actin-binding proteins, enzymes, signaling components and epigenetic modifiers (Figure 4), proteins involved in cellular transport and further proteins or RNAs representing other categories (Figure 5). We covered the feasibility of inhibition of these lncRNAs in the previous section.

Inhibition of transcription factors is feasible as demonstrated for drugs inhibiting the estrogen receptor and androgen receptor (179, 180). However, the transcription factors outlined in Figure 2 are difficult to target (181, 182). Proteolysis targeting chimeras might become game changers in this field. They are heterobifunctional structures consisting of a ligand binding to a protein to be degraded and a ligand for E3 ubiquitin ligase, resulting in proteasomal degradation of the target protein (183-186). In 2021 at least 15 targeted degraders will undergo clinical trials (187).

c-MET and IGF-1R (Figure 3) were identified as ESCC-related targets to be further validated with antagonizing monoclonal antibodies and small molecule tyrosine kinase inhibitors (188, 189). Up-regulated CCN B, D1 and E (Figure 3) function as activators of CDKs 1, 2, 4 and 6, which mediate critical mitotic functions (Figure 3) which also holds true for PLK1 (190-192). These targets are under further preclinical and clinical validation. The identified actin-binding proteins kindlin 2, LASP1 and FSCN1 (Figure 4) are associated with druggability issues. Further targets emerging from our search for further validation in ESCC are HDAC9, EZH2, KPNA2 and MDM2 (Figure 4 and Figure 5).

ESCC-specific targets were not identified by our search. Since we have focused on xenograft models for lncRNA and

target identification, immune-therapy related targets could not be identified. For further validation of the proposed therapeutic approaches proof-of-concept studies in patient-derived xenograft models and combination studies of inhibition of lncRNAS and chemotherapy and immune-related therapies would be helpful.

Conflicts of Interest

AN is and UHW was an employee of Roche.

Authors' Contributions

The Authors contributed equally to all aspects of the article.

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