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Stabilization of miRNAs in esophageal cancer contributes to radioresistance and limits efficacy of therapy

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Abstract

The five-year survival rate of esophageal cancer patients is less than 20%. This may be due to increased resistance (acquired or intrinsic) of tumor cells to chemo/radiotherapies, often caused by aberrant cell cycle, deregulated apoptosis, increases in growth factor signaling pathways, and/or changes in the proteome network. In addition, deregulation in non-coding RNA-mediated signaling pathways may contribute to resistance to therapies. At the molecular level, these resistance factors have now been linked to various microRNA (miRNAs), which have recently been shown to control cell development, differentiation and neoplasia. The increased stability and dysregulated expression of miRNAs have been associated with increased resistance to various therapies in several cancers, including esophageal cancer. Therefore, miRNAs represent the next generation of molecules with tremendous potential as biomarkers and therapeutic targets. Yet, a detailed studies on miRNA-based therapeutic intervention is still in its infancy. Hence, in this review, we have summarized the current status of microRNAs in dictating the resistance/sensitivity of tumor cells against chemotherapy and radiotherapy. In addition, we have discussed various strategies to increase radiosensitivity, including targeted therapy, and the use of miRNAs as radiosensitive/radioresistance biomarkers for esophageal cancer in the clinical setting.

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1.1 Introduction

Regardless of the best available treatment option, esophageal cancer is the sixth most leading cause of cancer-related death worldwide [1]. This could be due to the symbiotic interaction of tumor-associated macrophages and fibroblast cells, which promote desmoplastic reactions, manifesting in sterile inflammation and contributing to poor prognosis [2]. These types of cells normally enhance tumor hypoxia, expression of p38 MAPK, production of TGF- β and Wnt signaling in the tumor microenvironment. Once stabilized these pathways dysregulate expression and stability of various miRNAs and promote cancer progression. The dysregulated miRNAs disseminate epigenetic changes such as DNA damage, cell cycle arrest and effect sensitivity of tumor cells which are can to contribute to the resistance to therapy. Recent reports suggest that miRNA signatures can serve as biomarkers in predicting therapeutic outcome in a variety of solid tumors, including esophageal cancer [3]. Additionally, their stabilization in various tumor cells contributes to sterile inflammatory responses, which are refractory for immune or adjuvant therapies [3, 4]. Our previous studies [2, 5–7] have demonstrated the potential of radiotherapy to enhance T-cell responses against established and refractory tumors of the pancreas. Patients with esophageal tumors, like pancreatic tumors, often require the use of radiotherapy [3] in treatment regimens, and both external and internal beam radiation therapy are used to inactivate the tumor mass prior to surgical resection (also called ablative therapy). However, abscopal reactions and bystander toxicities often contribute to the intrinsic or acquired recurrence of cancer in ~30% of patients as compared to ~3% of patients who receive only chemotherapy [6]. Therefore, understanding the molecular mechanisms associated with the development of radioresistance in esophageal cancer is paramount in designing improved treatment modalities.

In the context of esophageal cancer, recent reports have demonstrated clinical correlations of sets of miRNAs with the outcome of radiotherapy, where expression of one set of miRNAs (discussed in detail and listed in Table 1) promote the development of radioresistance, while another set sensitizes esophageal cancer cells to radiation therapy treatments [8–19] Table 1 and Figure 1). Unfortunately, our knowledge in this regard is still rudimentary; therefore, a better understanding of the dynamic balance among these miRNAs is important for improving the therapeutic outcome in esophageal cancer patients.

In this review, we discuss key miRNAs and their contributions in dictating sensitivity and resistance for radiotherapy in esophageal cancer. We also focus on their potential utility as biomarkers (diagnostic and prognostic), and as therapeutic targets for the improved treatment of esophageal cancer.

2.1 miR-21

miR-21 is located on the plus strand of chromosome 17q23.2 within a coding area of the transmembrane protein 49 gene [4]. It is one of the most commonly implicated miRNAs in cancer [3, 20] and has been reported to be elevated in several cancers, including esophageal, breast, pancreatic, lung, glioblastoma, and neuroblastoma [21]. In esophageal cancer tissues,

increased levels of miR-21 (~7.0-fold) are significantly associated with advanced pathological stage, including lymph node metastasis and poor prognosis [22, 23].

Huang et al. (2013) [8] by using qRT-PCR found that miR-21 was up-regulated by ~1.3-fold (Figure 1, Table 1) by treatment with irradiation at 60 Gy in TE-R60 (radioresistant esophageal squamous cancer cells) esophageal squamous cell carcinoma (ESCC)-radioresistant cells relative to that of the TE-1 parental resistant ESCC cells [8]. They further observed that depletion of miR-21 by anti-miR-21 lentiviral transduction rendered TE-1 cells more sensitive to radiotherapy treatments compared to cells treated with a scrambled control anti-miR-21 [8]. To better understand the cause of radiosensitivity it was found that down-regulation of miR-21 led to apoptosis, perturbation of the cell cycle, and proliferation of TE-1 cells [22, 23]. miR-21 is known to target several cancer-signaling pathway molecules that control cell survival and apoptosis, including p53, Ras, and PI3K/Akt [24], which represent targets for radiosensitivity. In ESCC, suppression of miR-21 in TE-1 cells also led to downregulation of pAkt, which is downstream of the phosphatase and the tensin homolog (PTEN) signaling pathway. Furthermore, it was observed that PTEN was up-regulated in TE-1 cells treated with anti-miR-21 compared to cells treated with a scrambled control, but there was no significant change in PTEN mRNA. This suggests that there is negative correlation between the expression of miR-21 and PTEN protein in TE-1 cells. PTEN is a tumor suppressor that plays important roles in cell proliferation, apoptosis, and DNA repair processes that are critical for cellular radiosensitivity. Thus, activation of PTEN by downregulation of miR-21 targeting is thought to enhance radiosensitivity in ESCC cells. Overall, elevated expression of miR-21 increases the radioresistance of ESCC cells through the PTEN and PI3K/Akt pathways (Figure 2), consistent with previous studies on miR-21 in neuroblastoma and gastric tumor cells [25]. Thus, miR-21 may be explored as a potential prognostic biomarker and target for ESCC radiosensitization; nonetheless, additional experimentation is required to determine its clinical significance in esophageal cancer.

2.2 miR-31

miR-31 is located in the fragile region of chromosome 9p21.3, which is often deleted in cancers [26]. Unlike miR-21, overexpression of miR-31 has been shown to promote cytotoxicity in tumor cells and its levels is often reduced in cancers [27, 28], perhaps due to deletions or translocation of the fragile location of miR-31 in the genome.

Lenon et al. (2012) [9] demonstrated, by using miRNA microarray analysis, that the expression of miR-31 was significantly reduced (~2.0-fold) (Figure 1, Table 1) in radioresistant esophageal OE33R cancer cells compared to parental radiosensitive OE33P cell lines [9]. They found that irradiation with 2 Gy significantly induced the expression of miR-31 in OE33P cells 6h post irradiation. Further, overexpression of miR-31 by pre-miR-31 in OE33R cells increased cell sensitivity to 2 Gy irradiation, suggesting a role of miR-31 in conferring radiosensitivity in esophageal cancer cells [9]. To better understand the mechanisms underlying the miR-31 mediated regulation of radiosensitivity, miR-31 was overexpressed in OE33R cells and surprisingly, it was found that DNA repair proteins such as PARP1, SMUG1, MLH1, RAD51L3, MMS19 were down-regulated in OE33R cells, implicating miR-31 in modulating radiosensitivity in ESCC cells.

Many studies have suggested that the aforementioned DNA repair proteins play vital roles in defense against radiation-induced DNA damage, such that their low expression enhances cellular radiosensitivity [29]. Given the role of miR-31 in radioresistance *in vitro*, the function of miR-31 was assessed in esophageal cancer patients undergoing radiotherapy [21]. It was found that the levels of miR-31 was significantly increased (~1.2-fold) in patients who responded well to radiotherapy compared to patients who responded poorly to such regimes [9], implicating miR-31 in modulating sensitivity to radiotherapy *in vivo*. However, the altered expression of miR-31 was independent of histological subtypes and lymph node status in esophageal cancer patients [21]. Further, it was observed that miR-31 significantly downregulated (~2.0-fold) DNA repair proteins in esophageal cancer patients who were sensitive to radiotherapy treatments (Figure 2). These results from cancer patients also suggested that miR-31 mediated radiosensitivity, perhaps by regulating the expression of DNA repair genes in ESCC cells. On the basis of such clinical data, further investigation is warranted to elucidate the potential role of miR-31 as both a predictive marker of therapeutic responses, and as a novel therapeutic target to enhance the efficacy of radiation therapy in ESCC patients.

2.3 miR-96

miR-96 maps to the long arm of chromosome 7q32.2, and has been implicated as an oncogene in a variety of cancers such as non-small cell lung, bladder, colorectal, and breast [30–32]. Esophageal cancer cell lines (TE-1, ECa-109, and EC-9706) express significantly higher amounts (~2.0-fold) (Figure 1, Table 1) of miR-96 compared to normal human esophageal epithelial cells (HECC). Similarly, miR-96 expression was ~3.0-fold higher in esophageal cancer tissues than the corresponding adjacent normal tissues of patients [10]. A significant correlation was noted between tumor volume and metastasis in miR-96 positive cancer patients, though no associations were observed between the ages or gender of patients with regard to miR-96 expression. To investigate the effects of miR-96 on tumor progression *in vivo*, Xia et al, (2014) [10] established nude mouse models and found that implantation of pcDNA-miR-96-ESCC cells resulted in increased tumor weights compared to an empty vector. Moreover, ectopic expression of miR-96 in TE-1 and ECa-109 esophageal cancer cells promoted cell proliferation compared to control plasmids in cells. Further, they found that miR-96-overexpressing esophageal cancer cells exhibited significantly decreased apoptotic rates compared to nonoverexpressing cells following 6 Gy irradiation, suggesting that miR-96 promotes radioresistance. Mechanistically, it was determined that miR-96 negatively regulated the expression of the RECK (**R**eversion inducing **C**ysteine-rich protein with **K**azal motifs) protein, which controls the expression of matrix metalloproteinase 9 (MMP9) and fosters cancer invasion and metastasis [10] in EC radioresistant cells (Figure 2). In normal cells, RECK serves as a negative regulator for MMP9 [33], and acts as a tumor suppressor [34–36]. By Western blot analyses, the authors found that RECK was ~5.0-fold higher than in normal cells compared to esophageal cancer cells, indicating that miR-96, in part, in down-regulating RECK in esophageal cancer cells, promoted radioresistance in esophageal cancer [10]. Taken together, the above findings suggest that miR-96 is involved in resistance to radiotherapy in esophageal cancer cells and as such, may provide a novel therapeutic target in the treatment of esophageal cancer and/or radiotherapy-resistant

patients. Further studies are needed in esophageal cancer patients to consider miR-96 as a useful biomarker.

2.4 miR-98

miR-98 is located on chromosome X and regulates cell migration and apoptosis in cells [12, 37, 38], and is down-regulated in breast [39], lung [40], ESCC [12] and hepatocellular cancers [41]. Jin et al. (2016) found via miRNA microarray expression analysis that miR-98 was downregulated (~5-fold) (Figure 1, Table 1) in acquired ESCC radioresistant cells (EC9706R) compared to the parental EC9706 ESCC line following treatment with 8 Gy irradiation [11]. Acquired radioresistant cells also showed enhanced colony-forming ability and increased proliferation compared to parental cells. Functional studies further demonstrated that the overexpression of miR-98 promoted apoptosis and inhibited cell migration of ESCC cells [11]. miR-98 precursor-transfected cells showed significantly decreased survival following irradiation compared with the negative control cells, reflecting increased radiosensitivity, while miR-98 inhibition reversed the sensitivity of cells toward radiotherapy. Additionally, miR-98 overexpression significantly increased X-ray-induced apoptosis in radioresistant ESCC cells relative to non-transfected cells and negative control mimics. Moreover, the authors demonstrated that miR-98 overexpression significantly increased the expression of caspase-3 and decreased the expression of *BCL-2* in radiation-resistant esophageal cancer cells (Figure 2). In contrast, miR-98 inhibition had no such effects on the levels of caspase-3 and *BCL-2* in radioresistance cells following treatment with 8 Gy of irradiation. The authors concluded that miR-98 enhanced X-ray irradiation-induced apoptosis in esophageal cells by regulating the expression of apoptosis-related proteins. Thus, the down-regulation of miR-98 appears to render ESCC cells more resistant to radiation treatment and hence it may be considered as a therapeutic target by reversing its expression level in esophageal cancer cells.

2.5 miR-124

miR-124 is located on chromosome 8 [42] and is significantly reduced in a variety of cancer cells such as breast [43], colorectal [44], and esophageal [14], and acts as a tumor suppressor. Interestingly, upregulation of miR-124 predisposed glioma and colorectal tumor cells to radiotherapy [45, 46]. To explore the significance of miR-124 in esophageal cancer, Zhang et al. (2016) [13] examined the expression of miR-124 in 18 paired esophageal cancer tissues, and found that the expression of miR-124 was significantly reduced (~2.0-fold) (Figure 1, Table 1) compared to adjacent control tissues [13]. To investigate the physiological role(s) of miR-124 in esophageal cancer, cell counts and wound healing assays were performed in TE-1 esophageal cancer cells. The authors found that ectopic expression of miR-124 in TE-1 cells markedly inhibited the proliferation and invasion of cells compared with NC-mimic controls. To investigate the role of miR-124 in radiosensitivity, Zhang et al, (2016) [13] overexpressed miR-124 in TE-1 cells exposed to 8 Gy of irradiation, and found that ectopic expression of miR-124 followed by radiotherapy led to a higher percentage of apoptotic cells compared to control scrambled-treated cells. In order to elucidate the molecular mechanism(s) by which miR-124 enhances apoptosis in TE-1 cells following irradiation, the authors identified cyclin-dependent kinase 4 (CDK4) as a target of

miR-124. Further, it was found that miR-124 mimics significantly inhibited the luciferase activity of a reporter gene containing the wild-type 3' UTR of CDK4 but did not affect the luciferase activity of the reporter gene containing the mutant 3' UTR of CDK4. Western blot analysis confirmed that the overexpression of miR-124 decreased CDK4 protein levels (Figure 2). CDK4 is a member of the cell-cycle-related subfamilies, reported to participate in a variety of biological processes including cell proliferation and apoptosis [47]. Previously, it was demonstrated that overexpression of CDK resulted in a poorer prognosis and conferred resistance of glioma cells to treatment with irradiation [48]. These results suggest that increased expression of miR-124 leads to lower expression levels of CDK4, which enhances apoptosis of esophageal cancer cells induced by ionizing radiation (Figure 2). Collectively, the data suggest that miR-124 and CDK4 are promising radiosensitivity targets in the treatment of esophageal cancer.

2.6 miR-205

miR-205 is located on chromosome 1q.32.2 and is involved in development, proliferation, and apoptosis [49]. Recently, Pan et al. (2017) demonstrated that miR-205 was significantly upregulated (~10.0-fold) in radioresistant ESCC cells and its depletion increased radiosensitivity in the same cells [15]. Upregulation of miR-205 was also shown to enhance cell survival, DNA damage repair, and resistance to apoptosis, most interestingly in the epithelial-mesenchymal transition (EMT) post radiotherapy [15]. Other studies have reported that miR-205 induced radioresistance by targeting zinc-finger E-box binding homeobox 1 (ZEB1) in breast cancer [50]. miR-205 contains three putative Sp1 transcription factor-binding sites in its promoter region, which in response to radiation, increased the transcription of miR-205 and promoted radioresistance in ESCC (Figure 2). These results demonstrated that Sp1 could be phosphorylated by high dose radiation that in turn elevated its transcriptional activity [51, 52]. miR-205 can directly bind to the 3'-UTR of PTEN suppressing its expression and conferring radioresistance in cells (Figure 2). Subsequently, it was proposed that changes in the expression of miR-205 can modulate the activity of the AKT protein at serine 472 in parental cells, while dictating the fate of cells to treatment with radiation. It was noted that PTEN overexpression decreased DNA repair in miR-205-overexpressed cells and promoted radiation-induced cell apoptosis [15]. Hence, these data suggest that expression of miR-205 is mediated by the Sp1 transcription factor, which in turn promotes radioresistance through the PTEN/AKT (PI3K/AKT) pathway in ESCC (Figure 2). Thus, miR-205 may provide a potential biomarker of prognosis, and/or a critical determinant of radioresistance in patients, and it is anticipated that a miR-205-targeted approach may improve therapeutic outcome for esophageal cancer patients.

2.7 miR-381

The miR-381 is located on chromosome 14q32.31 and found to play a potential role in the development of radioresistance in esophageal cancer. [16] Lee et al. (2011) discovered that upregulation (~7.5-fold) of miR-381 inhibited the colony-forming capacity of malignant mast cells [53] and suppressed the proliferation and the invasive capacity of renal cancer cells [54, 55]. However, in glioblastoma, miR-381 acts as an oncogene to activate tumor growth [56, 57].

Recently, it was found that miR-381 was significantly downregulated (~3.0-fold) in esophageal cancer patients undergoing radiotherapy treatments compared to individuals who were not exposed to radiotherapy [16]. Similarly, the authors observed that radioresistant KYSE450 esophageal cancer cells expressed (~2.5-fold) less miR-381 compared to radiosensitive TE-1 cells [16]. Subsequent experimental evidence suggested that overexpression of miR-381 in radioresistant KYSE450 cells significantly decreased survival and migration capacity of these cells, supporting a negative functional role of miR-381 in the aggression of ESCC cells. Accordingly, inhibition of miR-381 led to increased survival and conferred an aggressive phenotype with an increased capacity for proliferation and migration. Consistent with this, KYSE450-miR-381 tumors grew slower than KYSE450-control tumors following irradiation of mice with 5 Gy irradiation, demonstrating their radiosensitive nature [16]. As expected, this effect was abrogated by overexpression of miRNA-381 in cells. Surprisingly, overexpression of miR-381 also inhibited tumor growth and was mediated by down-modulation of various signaling factors such as Catenin Beta 1 (CTNB1), Lymphoid Enhancer Binding Factor 1 (LEF1), Cyclin Dependent Kinase 1 (CDK1), X-Linked Inhibitor Of Apoptosis (XIAP), and C-X-C Motif Chemokine Receptor 4 (CXCR4), which are involved in tumor development (Figure 2). Moreover, Zhou et al, [58] found that XIAP is a direct target of miR-381 and binds with its 3'UTR region, dysregulating the enhanced expression of XIAP resulting in increased apoptosis, growth inhibition and radiosensitivity in ESCC cells. The XIAP protein belongs to the family of endogenous inhibitors of apoptosis (IAP) proteins that were solely responsible for disengaging cell death in various cell types by inhibiting distinct caspases residing at the terminal caspase cascade. It was also found that in the presence of a specific sequence, the Internal Ribosome Entry Site (IRES) residing at the 5' UTR region of XIAP was responsible for XIAP-translation via a cap-independent mechanism [59]. This provides evidence that the expression of XIAP is a requirement of a cell as it modulates various distinct biological processes especially regulating the caspase cascade within the cell. Therefore, the miR-381/XIAP pathway may be considered for elucidating therapeutic benefits, especially against the developed radioresistance in ESCC [59].

2.8 miR-17-5p

miR-17-5p, also known as miR-91, is located on chromosome 13q31, a genomic locus that often undergoes loss of heterozygosity in different types of cancer [60]. Previous studies suggested that miR-17-5p acts both as a tumor suppressor and promoter, depending upon the cellular environment and the target mRNAs expressed [61–64]. It is downregulated in cervical [65] and non-small cell lung carcinoma [66], while it is upregulated in bladder [67], breast [68], gastric [69], colorectal [70], hepatocellular [71] pancreatic [72] and esophageal cancer [17].

Non-obese severe combined immunodeficiency (NOD-SCID) mice transplanted with human esophageal adenocarcinoma (EAC) radioresistant OE33R cells, support the development of tumors in comparison to mice transplanted with isogenic radiosensitive OE33P cells. OE33R-bearing mice developed tumors faster and had larger tumor volumes than those in the OE33P-bearing group. A reason for the larger tumor volume may include the higher expression of cancer stem-like cell (CSC) marker genes *aldehyde dehydrogenase 1*

(ALDH1) and β -catenin, and reduced expression of miR-17-5p [17]. These genes are implicated in the regulation of CSCs in a number of solid tumors such as breast [73], lung [74] and prostate [75]. Further, it was identified that ALDH1+ve cells isolated from OE33R cells showed significantly higher resistance to 2 Gy radiation compared to ALDH1-ve populations, and the ALDH1+ve cells showed a higher survival (~1.2-fold) following irradiation with 2 Gy compared to ALDH1-ve cells [17]. It was also found that OE33R ALDH1+ve cells expressed ~2-fold lower levels of miR-17-5p compared to OE33R ALDH1-ve cells. The transient overexpression of miR-17-5p in OE33R rendered the cells more sensitive to a 2 Gy radiation dose compared to scrambled controls, further suggesting a role for miR-17-5p in radioresistance in EAC cells [17]. Several studies have highlighted the roles of mutations and genomic rearrangements in the focalization of EAC [76, 77], suggesting that genomic alterations may be responsible for decreased miR-17-5p expression.

Overexpression of miR-17-5p downregulates the expression of the *protein kinase CAMP-activated catalytic subunit beta (PRKACB)* and *Chromosome 6 Open Reading Frame 120 (C6orf120)* genes (Figure 2). C6orf120 has been demonstrated to induce endoplasmic reticulum stress-associated apoptosis in CD4(+) T cells, suggesting an immuno-regulatory function for this protein [78]. Recently, studies [2, 5–7] have demonstrated an anti-tumor impact of low dose radiation (LDR) in both murine and human models of gastric tumors where iNOS+ macrophages were indispensable for tumor infiltration, particularly of CD8+ T-cells, and subsequent tumor vasculature normalization and tumor immune destruction. Therefore, we anticipate that LDR may have modulated the expression of a set of miRNAs for adjusting the immune response against tumors. It is also anticipated that LDR-directed changes in the expression of a set of miRNAs may also account for both the radiosensitivity and immunogenicity of irradiated tumor cells via their effective uptake and clearance by the cells of the innate immune system. Compelling evidence suggested that EAC tumors with decreased miR-17-5p expression and associated increased C6orf120 expression have a poor response to radiation via C6orf120-mediated immunosuppression of CD4 (+) T cells. However, more work is required to elucidate the mechanism(s) of this resistance to neoadjuvant radiation therapy. These findings suggest that miR-17-5p may serve as a prognostic marker and may assist in predicting responses to neoadjuvant radiotherapy in EAC. In addition, miR-17-5p may provide a novel therapeutic target to increase the efficacy of radiotherapy for esophageal cancer patients.

2.9 miR-193a-3p

miR-193a-3p located on chromosome 17q11.2 has been implicated in a number of roles in different types of cancers. For example, it has been found to act as a tumor-suppressor in gastric cancer [79], renal cell carcinoma [80], hepatocellular carcinoma [81], and ovarian cancer [82], whereas it has an oncogenic role in esophageal squamous cell carcinoma [18].

Meng et al, 2015 showed that miR-193a-3p expression is substantially elevated (~192fold) in the radioresistant KYSE410 cell line compared to the sensitive KYSE150 line [18]. In addition, by colony formation and MTT assays, they found that inhibition of miR-193a-3p expression caused increased drug-induced cell death in KYSE410 cells. Moreover, transfection with an miR-193a-3p antagomiR in KYSE410 cells led to an accumulation of

gamma-H2AX, a well-known marker of double-strand breaks, and at the same time the levels of pro-apoptotic markers increased [18]. Additionally, after X-ray treatment, the survival of KYSE410 cells with down-regulated miR-193a-3p expression was lower than that of the control groups. These results suggest that miR-193a-3p may promote chemo- and radio-resistance in oesophageal squamous cells and play an important role in ESCC. Further, Meng et al, (2016) [18] have demonstrated that miR-193a-3p downregulates the expression level of the *PSEN1* gene. PSEN1 is an important component of γ -secretase and sensitizes cancer cell to chemo/radiotherapy [18]. The loss-of-function of PSEN1 allows an unresponsive DNA damage response and provides ESCC cells to attain chemo- and radioresistance [18]. Previously, Deng et al., (2015) also reported that miR-193a-3p through PSEN1 regulates the multichemoresistance of bladder cancer [83]. Together, the above findings highlight an important role for miR-193a-3p in inducing chemoresistance and radioresistance in ESCC cells. Further study is needed in human samples to verify the potential clinical applications.

2.10 miR-338–5p

miR-338–5p is located on chromosome 17q25.3 and often originates from an intron of the gene encoding the apoptosis-associated tyrosine kinase (AATK) [84]. It has been reported to be correlated with the carcinogenesis and progression of several human cancers, including colon [85], glioma [86], glioblastoma [87], hepatocellular [88] and esophageal squamous cell carcinoma [19].

In the case of ESCC, miR-338–5p was found to be significantly downregulated (~5.1-fold) in ESCC radioresistant TE-4R cells compared to the sensitive parental TE-4 ESCC cells [19]. It was observed that TE-4 cells transfected with an miR-338–5p mimic were more sensitive to treatment with 4 Gy radiation compared to the cells transfected with a control miRNA [19]. Cell viability analysis by MTS assays at different time points after irradiation with 4 Gy showed that TE-4 viability was significantly lower at day 5, and markedly lower on day 7 after irradiation [19]. These data clearly indicate that ectopic expression of miR-338–5p renders ESCC cells more sensitive to radiotherapy. Through western blot analysis, it was found that the miR-338–5p induced the expression of pro-caspase genes such as PARP and caspase-3, thus enhancing apoptosis in radio-exposed ESCC cells [19], a major factor regulating radioresistance in ESCC [89].

To further advance the understanding of the mechanism(s) associated with miR-338–5p induced apoptosis, Park et al (2017), by using bioinformatics identified the *survivin* gene as one of the targets of miR-338–5p, which was later also confirmed through luciferase-reporter assays [19]. It was also discovered that miR-338–5p downregulation enhanced the expression of survivin in the radioresistant cell line compared to the parental cells [19]. Survivin is a 16.5 kDa protein member of the IAP protein family, which is known for its inhibitory effect on caspases such as caspase 3, caspase 7 and caspase 9, and develops a negative regulation of apoptosis [90, 91]. Survivin is generally expressed and functions in the G2/M phase of the cell cycle, where at the initiation of mitosis, it interacts specifically with microtubules (γ -tubulin), and with GTP molecules provides stability to the spindle apparatus of the cell, which may contribute to development of radioresistance in ESCC cells

[91]. To explore the *in vivo* effects of miR-338–5P on radiosensitivity, xenografts were generated in mice using TE-4 cells. It was found that injection of miR-338–5p into the tumor mass increased the sensitivity to radiation therapy compared to control tumors [19]. Additionally, survivin expression was downregulated in the miR-338–5p transfected xenografts of mice.

Overall, the above findings reveal that downregulation of miR-338–5p plays a critical role in the development of radioresistance in esophageal cancer. Consistent with this, ectopic overexpression of miR-338–5p was shown to induce apoptosis and sensitivity to radiation treatment by interfering with survivin expression in cells, which is a known inhibitor of apoptosis. Therefore, miR-338–5p and survivin together may act as potential biomarkers, and may be explored as target molecules to enhance sensitization of ESCC cells to radiotherapy.

3.1 Clinical Perspectives

Alterations in the expression of both genetic and cellular components have important roles in dictating the fate of tumors following various therapeutic modalities. In this context, we propose that miRNAs may be important factors in facilitating such alterations in esophageal cancer. Of particular note, molecular targeting of various miRNAs in conjunction with neo-adjuvant therapy may provide a novel approach for restoring immunity against refractory tumors. Cited literature also supports our claim that modulating microRNA levels in tumor patients is likely to offer sensitization in refractory tumors as well enhancing the immune response. Since every individual inherits their own microRNA signature, microRNA-based therapeutics may become an important component of personalized medicine in the future for enhancing therapeutic outcome, not only against tumors, but also in inflammatory and metabolic diseases.

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Highlights of review

- Esophageal cancer is an aggressive form of carcinoma.
- miRNAs stimulate various signaling pathways in esophageal cancer resulting in tumor progression and inhibition.
- Modulation of miRNAs levels may provide a new therapeutic approach for esophageal cancer treatment.

Figure 1: Altered expression levels of miRNAs modulate radiotherapies in various human cancers.

The colored squares represent a relationship between miRNA (on the left) variation in response to radiotherapy in different cancer types (on the top).

Figure 2: Regulatory networks of various miRNAs associated with esophageal cancer. The schematic diagram depicts the putative cellular roles of miRNAs in the upregulation (upward arrows) or downregulation (downward arrows) of gene products in various cellular events involved in esophageal cancer pathogenesis.

Table 1:

Esophageal cancer-associated miRNAs and their reported biological functions in radioresistance and the affected pathways

miRNA	Genomic location	Approx Mean fold change in expression compared to controls	Tumor type	Radiation type (dose)	Property	Validation methods	Biological significance	Genes/ Proteins/ Pathways affected	Cell lines	References
miR-21	17q23.1	↑ 1.3	Esophageal Squamous cell carcinoma (ESCC)	X-ray Radiation (60 Gy)	Oncogenic	qRT-PCR; Western blot analysis; clonogenic Assay	Promotes cell proliferation, invasion and inhibits apoptosis	↓ PTEN; ↑ Akt; ↑ PI3K/AKT pathway	TE-1 cells	[8]
miR-31	9p21.3	↓ 2.0	Oesophageal Adenocarcinoma (OAC)	X-ray Radiation (2 Gy)	Tumor suppressor	qPCR; Microarray analysis; Western blot analysis; clonogenic Assay	Inhibits tumour growth and invasion	↑ PARP1; ↑ SMUG1; ↑ MLH1; ↑ MMS19	OE33 cells	[9]
miR-96	7q32.2	↑ 2.0	Esophageal Squamous cell carcinoma (ESCC)	X-ray Radiation (6 Gy)	Oncogenic	qRT-PCR; Western blot analysis;	Promotes cell proliferation, tumorigenesis, invasion, metastasis	↓ RECK; ↑ MMP9	HEEC, TE-1, ECa-109 and EC-9706 cells	[10]
miR-98	Xp11.22	↓ 5.0	Esophageal Squamous cell carcinoma (ESCC)	X-ray Radiation (8 Gy)	Tumor suppressor	qRT-PCR; Western blot analysis; Microarray analysis	Induces apoptosis and inhibits cell migration and invasion	↓ Bcl2; ↓ Bcl2 pathway; ↑ EZH2; ↑ caspase 3	TE1, ECA109, EC9706, KYSE30, KYSE150 and KYSE450 cells	[11, 12]
miR-124	8p23.1	↓ 2.0	Esophageal Squamous cell carcinoma (ESCC)	X-ray Radiation (8 Gy)	Tumor suppressor	qRT-PCR; Western blot analysis	Induces apoptosis and inhibits cell proliferation, invasion and metastasis	↑ CDK4; ↑ STAT3; ↑ CDK4 signaling pathway	TE-1 and Eca109 cells	[13, 14]
miR-205	1q32.2	↑ 10.0	Esophageal Squamous cell carcinoma (ESCC)	X-ray Radiation (6 Gy)	Oncogenic	qRT-PCR; Western blot analysis; clonogenic Assay	Inhibits apoptosis, induces Epithelial Mesenchymal Transition, metastasis	↑ Sp1; ↑ PTEN; ↑ miR-205/PTEN/PI3K/AKT pathway	KYSE30 and KYSE450 cells	[15]
miR-381	14q32.31	↓ 3.0	Esophageal Squamous cell carcinoma (ESCC)	X-ray Radiation (5 Gy)	Tumor suppressor	qRT-PCR; Microarray analysis	Inhibits cell proliferation, migration and invasion	↑ CTNNB1, ↑ LEF1, ↑ CDK1, ↑ XIAP, and ↑ CXCR4	TE1, ECA109, EC9706, KYSE30, KYSE150, and KYSE450 cells	[16]
miR-17-5p	13q31	↓ 3.0	Esophageal Adenocarcinoma (EAC)	X-ray Radiation (2 Gy)	Tumor suppressor	qPCR; clonogenic Assay	Induces apoptosis and inhibits tumorigenesis	↑ C6orf120; ↑ ALDH1; ↑ β-	OE33 cells	[17]

miRNA	Genomic location	Approx Mean fold change in expression compared to controls	Tumor type	Radiation type (dose)	Property	Validation methods	Biological significance	Genes/ Proteins/ Pathways affected	Cell lines	References
								catenin; ↑ PRKACB		
miR-193a-3p	17q11.2	↑ 192.0	Esophageal Squamous cell carcinoma (ESCC)	X-ray Radiation (8 Gy)	Oncogenic	qRT-PCR; clonogenic Assay	Inhibits apoptosis, Induces DNA damage,	↓ PSEN1	KYSE150, KYSE410, KYSE450 and KYSE510 cells	[18]
miR-338-5p	17q25.3	↓ 5.1	Esophageal Squamous cell carcinoma (ESCC)	X-ray Radiation (4 Gy)	Tumor suppressor	qRT-PCR; Microarray Analysis	Inhibits apoptosis	↓ PARP; ↓ Caspase 3; ↑ Survivin; ↑ mTOR signaling; ↑ Akt; ↑ PI3K; ↑ PI3K/Akt/P70S6K1 pathway	TE-4 and TE-4R cells	[19]