

# Exploring the Potential of MicroRNA Let-7c as a Therapeutic for Prostate Cancer

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**Prostate cancer (PCa) is one of the leading causes of mortality worldwide and often presents with aberrant microRNA (miRNA) expression. Identifying and understanding the unique expression profiles could aid in the detection and treatment of this disease. This review aims to identify miRNAs as potential therapeutic targets for PCa. Three bio-informatic searches were conducted to identify miRNAs that are reportedly implicated in the pathogenesis of PCa. Only hsa-Lethal-7 (let-7c), recognized for its role in PCa pathogenesis, was common to all three databases. Three further database searches were conducted to identify known targets of hsa-let-7c. Four targets were identified, HMGA2, c-Myc (MYC), TRAIL, and CASP3. An extensive review of the literature was undertaken to assess the role of hsa-let-7c in the progression of other malignancies and to evaluate its potential as a therapeutic target for PCa. The heterogeneous nature of cancer makes it logical to develop mechanisms by which the treatment of malignancies is tailored to an individual, harnessing specific knowledge of the underlying biology of the disease. Resetting cellular miRNA levels is an exciting prospect that will allow this ambition to be realized.**

## Prostate Cancer

Prostate cancer (PCa) is one of the most prominent causes of cancer-related mortality in developed countries and accounts for around 8% of all malignancies, affecting ~15% of the male population.<sup>1–3</sup> In the United Kingdom, PCa is the most common cancer in males, accounting for 26% of all new diagnoses.<sup>4</sup> It is characterized by slow growth, and the majority of patients survive >10 years, but there is also a substantial reduction in quality of life.<sup>2</sup>

The prostate-specific antigen (PSA) was first discovered in 1979 and has subsequently developed into a valuable marker not only in the diagnosis of the disease but also in monitoring treatment response and residual disease.<sup>5,6</sup> Screening for atypical levels of PSA within the serum has allowed for early detection of this disease and has led to a 20% decrease in mortality.<sup>7</sup> However, it is also associated with both over-diagnosis and under-diagnosis, as well as over-treatment of men with low-risk disease.<sup>7,8</sup>

The European Association of Urology (EAU) guidelines for the treatment of PCa include non-targeted and invasive treatments such as radical prostatectomy, radiotherapy, hormonal therapy, and chemo-

therapy.<sup>9</sup> Taxane-based therapeutics such as docetaxel have proved effective; however, constant efforts are made to overcome limitations of water insolubility<sup>10</sup> and drug resistance.<sup>11</sup> In 10%–15% of patients, the cancer presents as an aggressive form that is characterized by metastases in the lymphatic system and bone sites.<sup>12</sup> Androgen deprivation is the standard clinical therapy for advanced PCa and is employed to hinder further growth.<sup>2</sup> Androgen deprivation therapy is effective for 1–2 years, after which the disease becomes resistant and is known as castrate-resistant prostate cancer (CRPC).<sup>2</sup> For those with CRPC, the standard of care is palliative.<sup>2</sup>

It is therefore crucial to identify novel, non-invasive, potent therapies to overcome the limitations of the current standard of care.<sup>13</sup> As PCa is a heterogeneous disease, it is rational to consider utilizing molecular markers that can be both predictive and prognostic, as well as conferring stage-dependent, customized treatments.<sup>14</sup> This review, therefore, will explore the role of miRNAs in prostate cancer with a focus on Lethal-7c (Let-7c), which was identified through the literature and computational analysis as being highly downregulated in PCa. Further computational analysis will explore the identification of let-7c target genes and use therefore as a biomarker, as well as a therapeutic and prognostic agent.

## MiRNA Biogenesis

MiRNAs are a group of highly conserved, non-coding RNAs that are approximately 18–22 nt in length.<sup>1,15</sup> They operate post-transcriptionally by attaching to the 3' UTR of mRNAs via complementary base pairing, to instigate mRNA degradation and/or translational suppression.<sup>1</sup> Various cellular processes involve the action of miRNAs, including cellular proliferation and apoptosis.<sup>16</sup> The process of miRNA maturation and mechanisms of action are detailed in Figure 1.

## MiRs in Cancer

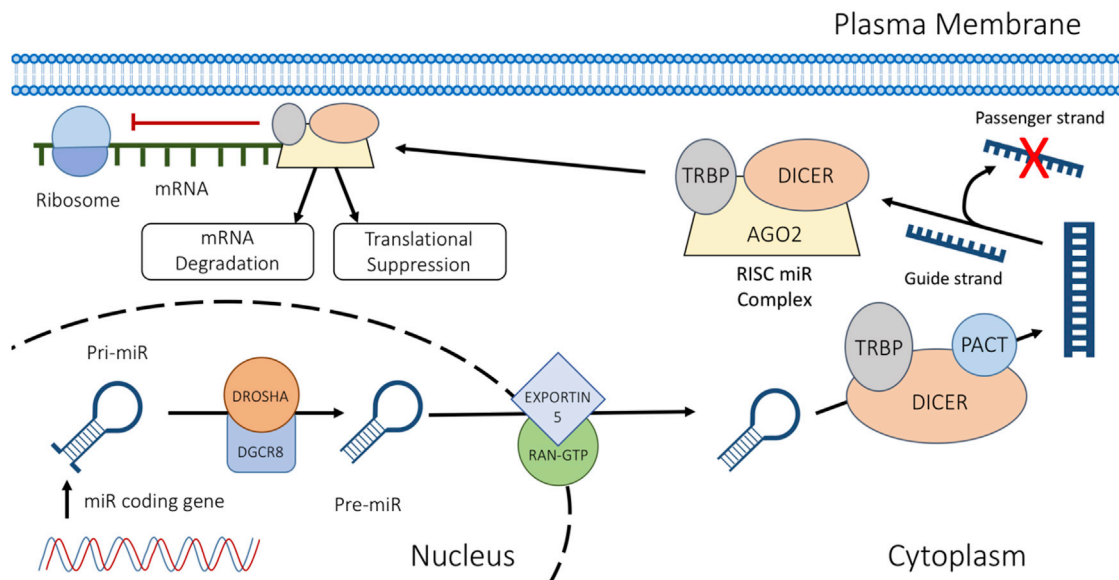
The human genome encodes for more than 1,917 miRNAs that can target multiple genes (<http://www.mirbase.org/>).<sup>17</sup> Around 40% of miRNA genes are either intronic or exonic and are situated within

<https://doi.org/10.1016/j.omtn.2019.09.031>.

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**Figure 1. miRNA Biogenesis**

The miRNA is transcribed by RNA polymerase II to form a pri-miRNA. The RNase III enzyme DROSHA cuts the single-stranded RNA/double-stranded RNA (ssRNA/dsRNA) junction to create a pre-miRNA. The pre-miRNA is transported to the cytoplasm via EXPORTIN 5/RAN-GTP. It then undergoes modification by DICER. The guide strand is then incorporated into the RNA-induced silencing complex (RISC), where it leads the complex toward target mRNA transcripts.

coding mRNAs.<sup>18</sup> Intergenic miRNAs are positioned in non-coding regions and are transcribed by unidentified promoters.<sup>19</sup> Around 50% of these genes are located in chromosomal sites that are susceptible to rearrangement or deletion in cancer.<sup>14,18,20</sup> Mutations in the stem region of miRNAs may affect the processing abilities of DGCR8 and DICER. However, mutations that occur in the loop region of pre-miRNAs do not affect processing capabilities.<sup>20</sup> Hanahan and Weinberg<sup>21</sup> suggested 10 essential modifications that occur within a cell's physiology that ultimately determine malignant growth: self-sufficiency in growth signals, insensitivity to growth suppressors, evasion of apoptosis, unlimited replicative potential, persistent angiogenesis, tissue invasion and metastasis, avoidance of immune destruction, genomic instability and mutation, deregulated cellular energetics, and tumor-promoting inflammation. Within all these processes, miRNA dysregulation can lead to advancing of the disease.

Novel miRNA targets are now being identified that may have a role in driving the oncogenic processes of PCa.<sup>22</sup> In identifying miRNAs involved in PCa pathogenesis, light may be shed upon pathogenic processes such as cellular proliferation, epithelial to mesenchymal transition (EMT), and the emergence of androgen independence.<sup>3</sup> Specific miRNAs have also been implicated in the development of chemoresistance in a number of malignancies, where modulation resulted in enhanced therapeutic sensitivity.<sup>23,24</sup> In PCa specifically, Liu et al.<sup>25</sup> showed that miR-34a plays a role in chemosensitivity to paclitaxel. This is achieved through regulation of the JAG1/Notch1 axis. The authors postulated therefore that miR-34a therapy could be used to overcome chemoresistance in PCa.

### MiRNA Expression in Prostate Cancer

Significant advancement has been made in the identification of miRNA target genes in cancer through validation using wet-lab experimentation. A literature search was performed using the miRCancer, Web of Science, and PubMed repositories for miRNAs associated with PCa. Table 1 presents a selection of aberrantly expressed PCa miRNAs, alongside associated targets identified from the literature.

### Comparison of Prostate Cancer-Associated MiRNAs

Computational methods for miRNA gene target identification are an auspicious tool to aid in building a complete picture of miRNA networks.<sup>26</sup> With a single miRNA having the potential to regulate such a wide variety of genes, it is often unrealistic to depend solely on wet-lab experimentation. Therefore, dry-lab approaches can be utilized as low-cost alternatives for the identification of miRNA in disease.<sup>27</sup> Three database searches were compared to identify PCa-associated miRNAs that were common to all. The three miRNA databases used were miRCancer, mir2Disease, and PhenomiR2.0.<sup>28–30</sup> Results of this search are included in Figure 2 and Table 2. Only hsa-let-7c was common to all three databases.

### The let-7 Family of MiRNAs in Cancer

Lethal-7 (let-7) was the second family of miRNAs discovered in *Caenorhabditis elegans* and was subsequently the first discovered in humans.<sup>31</sup> This family encompasses 13 members (let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, miR-98, and miR-202) that are spread across nine chromosomes.<sup>31</sup> Each of these members represents a different isoform of the let-7



**Table 1. miRNA Expression in PCa Alongside Respective mRNA Target Transcripts Identified in the Literature**

PCa miR	Expression (PCa versus Control)	Target	References
hsa-let-7c	down	MYC, RAS, HMGA2	47,48
hsa-miR-21	up	MARKS, PDCD4, TPM1	83
hsa-miR-32	up	BTG2	84
hsa-miR-34a	down	CD44, Cyclin D1, CDK4, CDK6, c-MET, ZEB1	85,86
hsa-miR-96	up	RAD51, REV1	87
hsa-miR-100	down	MYC, Oct4, KLF4	88
hsa-miR-105	down	CDK6	89
hsa-miR-124	down	TLN1	90
hsa-miR-125b	up	EIF4EBP1, p14ARF	91
hsa-miR-130b	down	MMP2	92
hsa-miR-133b	down	EGFR	93
hsa-miR-141	up	SHP	94
hsa-miR-143	down	GOLM1	95
hsa-miR-146a	down	Rac1	96
hsa-miR-153	up	PTEN, FOXO1	97
hsa-miR-154	down	CCND2	98
hsa-miR-155	down	STAT3	99
hsa-miR-181b	up	MLK2	100
hsa-miR-181c	up	DAX-1	101
hsa-miR-181d	up	DAX-1	101
hsa-miR-182	up	NDRG1	102
hsa-miR-183	up	DKK-3, SMAD4	103
hsa-miR-187	down	ALDH1A3	104
hsa-miR-200b	down	ZEB1, ZEB2	105

MARKS, myristoylated alanine-rich protein kinase C substrate; MMP, matrix metalloproteinase; NDRG1, N-myc downstream regulated gene 1; PTEN, phosphatase and tensin homolog; SHP, small heterodimer partner; TLN1, talin 1; TPM1, tropomyosin 1; ZEB, zinc-finger E-box binding homeobox.

gene.<sup>32</sup> These highly conserved miRNAs have been implicated as mediators of cellular differentiation throughout the development of different species, from nematodes to *Homo sapiens*.<sup>31</sup>

The let-7 genes are commonly found in regions that are altered or deleted in human malignancies, signifying a potential role as tumor suppressors.<sup>33</sup> Lin28A and Lin28B, two closely related RNA binding oncogenes that have a prominent role in suppressing let-7 biogenesis, are upregulated in >15% of malignancies.<sup>34</sup> Lin28 attaches to the hairpin precursor of pri-let-7 and pre-let-7, and blocks DROSHA-mediated processing of pri-let-7.<sup>35</sup> This appears to be very selective for the let-7 family because this phenomenon is not observed in other miRNAs.<sup>35</sup>

Interestingly, Zhou et al.<sup>36</sup> validated claims of significantly higher levels of Lin28B in lung cancer tissues and established a link between

miR-203, Lin28B, and let-7. The authors showed that overexpression of miR-203 was followed by significant upregulation of let-7. Moreover, apoptosis was promoted as a result of miR-203-induced suppression of Lin28B and let-7 biogenesis.<sup>36</sup>

Targeting Lin28B in acute myeloid leukemia (AML) inhibited proliferation and induced cell-cycle arrest.<sup>37</sup> Conversely, overexpression of Lin28B stimulated proliferation in AML.<sup>37</sup> Lin28B was also found to be correlated with MYC, a prominent driver of multiple myeloma (MM) progression.<sup>38</sup> Manier et al. demonstrated that MYC expression is under the control of the Lin28B/let-7 axis, and that induced overexpression of let-7 repressed tumor growth by mediating the expression of MYC. Chang et al.<sup>39</sup> then demonstrated that MYC prompts Lin28B expression in many human and mouse tumors (Figure 3).

### The Role of let-7 in Cancer Stem Cells

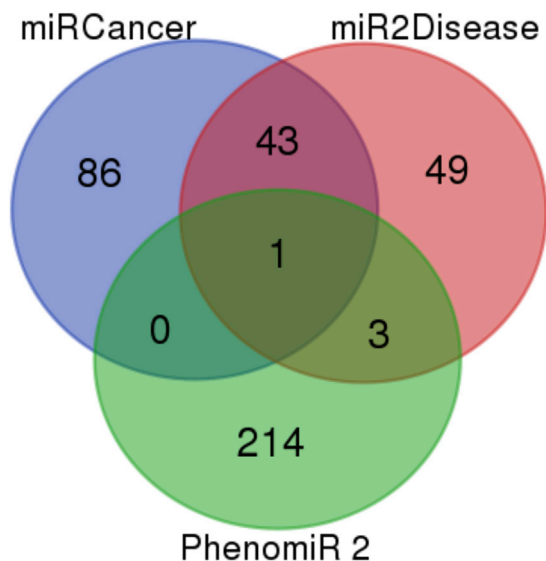
Embryonic stem cells continually replicate and divide, differentiating into organs under the guidance of transcription factors at certain points in time.<sup>40</sup> Conversely, adult stem cells exist in a passive state, waiting for environmental cues such as tissue damage before the activity is stimulated.<sup>40</sup> Cancer stem cells (CSCs) are a rare population that have similar characteristics to regular stem cells, yet can resist treatment and act as a source of tumor recurrence.<sup>41</sup>

Prostate CSCs exhibit aberrant gene expression patterns and can be used in identification and targeting.<sup>40,42</sup> Although surgical resection is a viable treatment option in PCa, it does not ensure that the disease has been eradicated, because thousands of cells may have escaped into the bloodstream as the lesion ruptures the basal lamina.<sup>43</sup> Any CSCs within this population have the capabilities of interacting with other microenvironments to safeguard survival and propagate recurring neoplasms.<sup>43</sup>

The survival of CSCs may be dependent on post-transcriptional gene regulation, including that carried out by let-7.<sup>44</sup> Lin28A and Lin28B are highly expressed within stem cells, where they suppress let-7 biogenesis in embryonic stem cells and CSCs to preserve stem cell characteristics.<sup>34,38,44</sup> Lin28 suppression and subsequently let-7 upregulation are correlated with a less aggressive phenotype in PCa.<sup>45</sup> Acquired resistance to a number of taxanes and platinum-based cancer therapeutics is also a product of the deregulated Lin28/let-7 axis.<sup>34</sup> Not surprisingly, Lin28 expression correlates to a poor prognosis.<sup>34</sup> Albino et al.<sup>46</sup> forced the overexpression of Lin28A and Lin28B in RWPE-1 cells (normal prostate epithelial cell line). They found significantly higher colony formation in soft agar, suggesting that both Lin28A and Lin28B endorsed tumorigenic and CSC activity.<sup>46</sup>

### The Role of let-7c in Prostate Cancer

Let-7c functions as a tumor suppressor and is downregulated in PCa.<sup>47</sup> Downregulation of let-7c has a positive effect on cellular proliferation and anchorage-independent growth of PCa cells *in vitro*, whereas overexpression of let-7c induced the opposite effect.<sup>47</sup> The



**Figure 2. A Venn Diagram Displaying Database Analysis Results Using miRCancer, miR2Disease, and PhenomiR2 Databases**

One target, hsa-let-7c, was common to all three databases.

androgen receptor (AR) plays a crucial role in PCa pathogenesis and the progression to castrate resistance following androgen deprivation therapy.<sup>48</sup> Let-7c was shown to regulate AR transcription via MYC.<sup>48</sup> PCa specimens reveal that AR expression is positively correlated to Lin28 expression and negatively correlated to let-7c expression.<sup>48</sup> Interestingly, let-7c suppression improved the ability of androgen-sensitive PCa cells to grow in an androgen-deprived environment *in vitro*.<sup>47</sup> Table 3 summarizes let-7 expression profiles in a host of malignancies.

#### Let-7c Targets

A computational search was conducted to identify let-7c target genes that are common to three databases: miRSearch 3.0, miRsel (validated and predicted), and starBase 2.0 (predicted).<sup>49–51</sup> The results of this search are included in Figure 4 and Table 4. The four hsa-let-7c targets common to all three databases include HMGA2, MYC, TNFSF10, and CASP3.

#### HMGA2

High mobility group, A2 (HMGA2) is a long-established target of the let-7 family.<sup>52</sup> The 3' UTR of HMGA2 contains many potential let-7 binding sites and is suppressed during let-7 upregulation in embryogenesis.<sup>31</sup> According to the reversed embryogenesis model, HMGA2 levels should be primarily upregulated in tumorigenesis.<sup>53</sup> HMGA2 expression in PCa correlates with this model because the HMGA2 expression was found to be significantly higher in PCa tissues than in normal prostate tissues.<sup>54</sup>

Cancer cells share three main characteristics with embryonic cells: proliferation, invasion, and immortality.<sup>55</sup> Epithelial to mesenchymal transition (EMT) is a mechanism that occurs during embryonic

**Table 2. Database Analysis Results Using Predictive Databases**

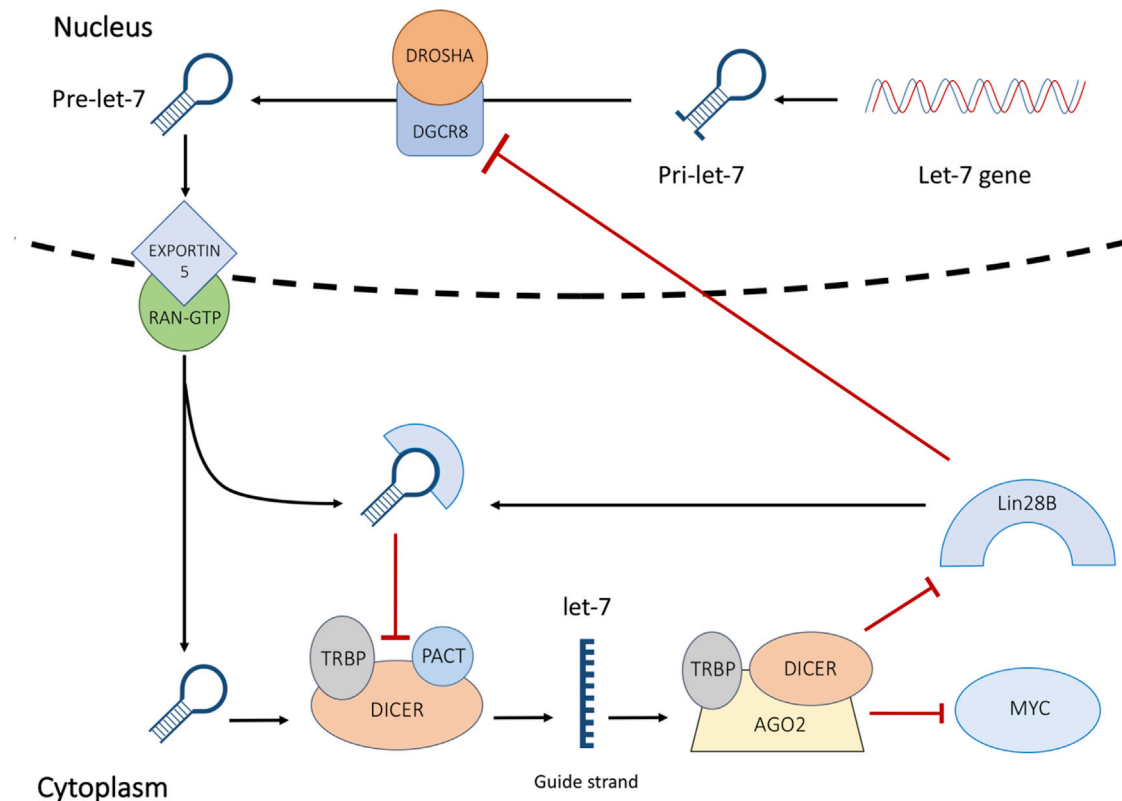
Database	Shared miRNAs	Total
miRCancer miR2Disease PhenomiR 2.0	hsa-let-7c	1
	hsa-miR-146a; hsa-miR-16; hsa-miR-125b	
	hsa-miR-22; hsa-miR-503; hsa-miR-222	
	hsa-miR-221; hsa-miR-195; hsa-miR-96	
	hsa-miR-27a; hsa-miR-23b; hsa-miR-101	
	hsa-miR-31; hsa-miR-143; hsa-miR-199b	
	hsa-miR-330; hsa-miR-449a; hsa-miR-223	
	hsa-miR-141; hsa-miR-194; hsa-miR-30c	
miRCancer miR2Disease	hsa-miR-224; hsa-miR-205; hsa-miR-103	43
	hsa-miR-24; hsa-miR-100; hsa-miR-182	
	hsa-miR-30b; hsa-miR-345; hsa-miR-25	
	hsa-miR-23a; hsa-miR-181b; hsa-miR-21	
	hsa-miR-15a; hsa-miR-20a; hsa-miR-183	
	hsa-miR-145; hsa-miR-26b; hsa-miR-497	
	hsa-miR-218; hsa-miR-26a; hsa-miR-34a	
	hsa-miR-29b	
mir2Disease PhenomiR 2.0	hsa-let-7d; hsa-let-7g; hsa-let-7b	3

development that allows for tissue morphogenesis.<sup>55</sup> However, evidence now suggests that EMT arises in malignant cells via signals from the tumor microenvironment, leading to metastatic potential.<sup>55</sup> During EMT, epithelial cells lose characteristic features and adopt features that are typical of mesenchymal cells.<sup>56</sup> Epithelial (E)-cadherin is a distinctive feature of epithelial cells, with expression lost during the transition to a mesenchymal phenotype.<sup>56</sup> Watanabe et al.<sup>57</sup> highlighted the responsibility of HMGA2 in reversibly maintaining the EMT process in pancreatic cancer. An inverse correlation exists between HMGA2 and E-cadherin expression because HMGA2 directly activates SNAIL, a transcriptional repressor of E-cadherin.<sup>57</sup> Suppression of HMGA2 in PC3 and DU145 PCa cells caused a marked reduction in proliferation, cell migration, and invasion, as well as diminishing the occurrence of EMT in both cell lines.<sup>58</sup>

#### MYC

There are three members in the Myc family, c-Myc (MYC), n-Myc (MYCN), and l-Myc (MYCL).<sup>59</sup> MYC is a proto-oncogene that encodes for a transcription factor that mediates cellular proliferation, growth, and apoptosis.<sup>60</sup> Aberrant expression of MYC is a common feature of cancer in humans.<sup>60</sup> MYC is frequently overexpressed in PCa<sup>61</sup> through the activation of the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways.<sup>62</sup> MYC suppression through JMJD1A knockdown in PCa cells inhibited cell proliferation and cell survival.<sup>63</sup>

The role of MYCN has been reported alongside HMGA2, Lin28B, and Let-7 in high-grade serous ovarian cancer.<sup>64</sup> Helland et al.<sup>64</sup> verified that overexpression of MYCN was correlated with amplification of



**Figure 3. Schematic Diagram of the Lin28B/MYC/Let-7 Interaction**

Let-7 maturity blocks the transcription of Lin28B and MYC. In the presence of Lin28B, pri-let-7 and pre-let-7 processing is inhibited. Repression of mature let-7 triggers an upsurge in MYC and Lin28B expression.

Lin28B and HMGA2, and inversely correlated with let-7 expression. MYC is also associated with the strong angiogenic promoter, vascular endothelial growth factor (VEGF).<sup>65</sup> Zhao et al.<sup>65</sup> demonstrated that VEGF drives the renewal of lung and breast CSCs via upregulation of MYC and SOX2. VEGF was found to trigger STAT3-mediated MYC expression and was ultimately associated with a poor prognostic outcome.<sup>65</sup>

#### **TNFSF10/Apo2L/TRAIL**

The tumor necrosis factor-related apoptosis-inducing ligand (TNFSF10/Apo2L/TRAIL) can trigger apoptosis when bound to receptors (TRAIL-R1/R2), preferentially targeting malignant cells.<sup>66</sup> Gang et al.<sup>67</sup> demonstrated the insensitivity of PC3 cells to TRAIL-induced apoptosis. Moreover, Anees et al.<sup>68</sup> discovered that 99.5% of 200 PCa tissue samples exhibited either reduced expression of pro-apoptotic TRAIL receptors, increased FLICE inhibitory protein expression, or both. Elevated TRAIL presence in the tumor microenvironment was also positively correlated with recurrence-free survival in PCa tissues.<sup>68</sup>

However, TRAIL/TRAIL-R has also been shown to promote metastasis through activation of PI3K and Rac1.<sup>69</sup> Intriguingly, Haselmann

et al.<sup>66</sup> discovered that TRAIL-R2 interacted with DROSHA and DGCR8 within the nuclei of pancreatic tumor cells. Upon investigation, they found that TRAIL-R2 inhibited DROSHA/DGCR8 activity, and subsequent knockdown of TRAIL-2 resulted in an upregulation of pri-let-7 processing and levels of mature let-7.<sup>66</sup> Mature let-7 levels were inversely correlated with Lin28B and HMGA2 levels, and thus cellular proliferation was inhibited.<sup>66</sup> Hartwig et al.<sup>69</sup> revealed how TRAIL is responsible for cytokine secretion in TRAIL-resistant tumor cells. CCL2 is a cytokine that promotes aggregation of the tumor-supportive M2 macrophage phenotype in the tumor microenvironment.<sup>69</sup> CCL2 and TRAIL presence correlated with M2 markers in lung adenocarcinoma patients.<sup>69</sup> This evidence suggests that TRAIL also has a cancer-promoting role in the tumor microenvironment.

#### **Caspase-3**

Caspase-3 (CASP3) is part of the caspase family, with a prominent role in mediating apoptosis upon cellular exposure to cytotoxic drugs and radiotherapy.<sup>70</sup> A large cohort study (N = 1,902) performed by Pu et al.<sup>71</sup> showed that there is a significant association between CASP3 expression and breast cancer-specific survival in breast cancer patients. An interesting study by Wang et al.<sup>72</sup> demonstrated that T47D breast cancer cells highly expressing Lin28B were much more



**Table 3. A Summary of the let-7 Expression Profile in Various Malignancies**

Cancer	Cell Line Tested	Expression	Reference
Breast	MCF10A-HER2/3, BT474, SKBR3, MCF10A-NeuNT, SUM159, SUM1315	↑ SHP2	106
		↑ ZEB1	
		↑ MYC	
		↑ Lin28B	
		↑ RAS	
		↓ Let-7	
Lung	NSCLC	↑ Lin28B	107
		↓ Let-7	
Multiple myeloma	MOLP-8, KMS12BM, RPMI8226, KMM-1	↑ Lin28B	38
		↑ MYC	
		↓ Let-7	
Neuroblastoma	BE(2)-C, SMS-KCNR, CHLA90	↑ Lin28B	108
		↑ MYCN	
		↓ Let-7	
Oral	OSCC	↑ Lin28B	109
		↑ ARID3B	
		↑ HMGA2	
		↑ OCT4	
		↑ SOX2	
Ovarian	ES-2	↓ Let-7	110
		↑ Lin28	
		↑ IGF2BP1	
		↑ HMGA2	
Pancreatic	PDAC	↓ Let-7	111
		↑ Lin28B	
		↓ SIRT6	
Prostate	PrEC, RWPE-1, LNCaP, DU145, PC3	↓ Let-7	46
		↑ Lin28A	
		↑ Lin28B	
		↓ ESE3/EHF	
		↓ Let-7	

ARID3B, AT-rich interactive domain 3B; IGF2BP1, insulin-like growth factor 2 mRNA-binding protein 1; NSCLC, non-small-cell lung carcinoma; OSCC, oral squamous cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; SIRT6, sirtuin 6.

resistant to radiation. As radiotherapy is known to trigger apoptosis in breast cancer, Wang et al.<sup>72</sup> investigated this effect on SK-BR-3 cells, discovering that stable Lin28B expression caused a significant reduction in radiation-induced apoptosis compared with that of the control. Furthermore, they noted lower levels of CASP3 activation in SK-BR-3 cells with stable Lin28B expression, signifying Lin28B as a mediator of the CASP3 apoptotic pathway.<sup>72</sup>

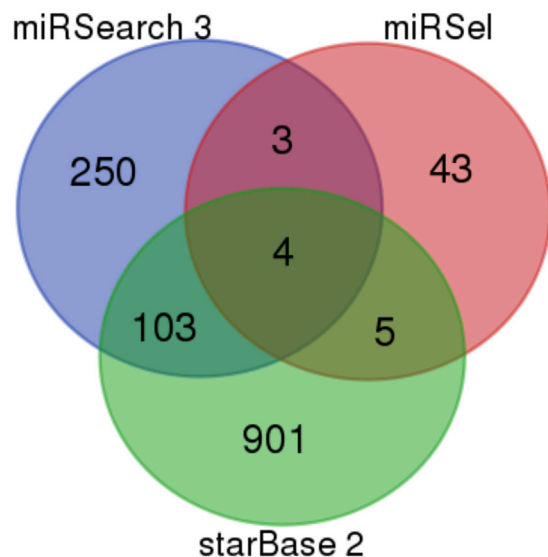
CASP3 expression was found to be significantly lower in moderately and poorly differentiated prostatic tumors when compared with well-differentiated prostate adenocarcinomas and normal prostate

specimens.<sup>73</sup> Furthermore, pro-CASP3 and cleaved CASP3 expression were assessed in radical prostatectomy samples as prognostic indicators.<sup>74</sup> Patients who presented with negative pro-CASP3 and cleaved CASP3 expression had a significantly worse prognosis.<sup>74</sup> Therefore, pro-CASP3 and cleaved CASP3 expression may serve to identify PCa patients at risk for disease progression.<sup>74</sup>

However, recent evidence supports the idea that CASP3 plays a role in tumor angiogenesis and tumor relapse. Zhou et al.<sup>70</sup> presented evidence showing that CASP3 knockout (CASP3KO) HCT116 colon cancer cells were significantly less invasive and exhibited increased sensitivity to radiation and the chemotherapeutic agent, mitomycin C. Furthermore, CASP3KO HCT116 cells showed reduced EMT characteristics by displaying higher E-cadherin expression and reduced SNAIL and ZEB1 expression compared with the control.<sup>70</sup> Figure 5 illustrates let-7 targets and the potential implications of target upregulation.

#### Modes of Let-7c Upregulation

The introduction of miRNA into tissues can come in many forms, for example, plasmid DNA that encodes for specific miRNA, or indeed antagomirs could be utilized for silencing expression.<sup>75</sup> For this, a plethora of delivery systems for genetic cargo is currently being used in research, of which viral vectors are most efficient.<sup>76</sup> For example, Nadiminty et al.<sup>47</sup> utilized a lentivirus system that encoded for a GFP-tagged let-7c precursor to explore its potential in PCa treatment. The treatment's efficacy was tested using xenografts in nude male mice injected subcutaneously with either C4-2B, PC456C, or DU145 cells. Once tumors had reached a size of 0.5 cm<sup>3</sup>, the mice were randomized into two groups. The experimental mice were treated with a single intratumoral injection (1 × 10<sup>7</sup> ifu) of let-7c encoding lentivirus, and control mice received a single injection of GFP encoding lentivirus. After 3 weeks the mice were sacrificed, and the tumors were excised for RNA isolation, to assess the levels of let-7c. It was seen that in all xenograft models injected with let-7c encoding lentivirus, a significant upregulation in gene expression (\*p < 0.05) was observed. Indeed, the mice treated with let-7c encoding lentivirus showed significant tumor growth inhibition in all cell line groups compared with the control. These findings propose that overexpression of let-7c can suppress PCa growth, making it an attractive therapeutic option to explore.<sup>47</sup> Yet, there are trepidations around the use of viral vectors as gene carriers due to toxicity, mutagenesis, and the hampered capacity for genetic cargo.<sup>76</sup> There are many examples of non-viral vectors that can be utilized for these applications, including cationic polymers, peptides, and liposomes, all of which exhibit the skill to package and deliver genetic cargo to the nucleus of cells.<sup>77</sup> Liposomes are made of a membrane composed of lipids, in which nucleic acids can be encapsulated. Liposomes come in three forms: cationic, neutral, and anionic. Cationic liposomes are the most frequently used form because of this heightened interaction with cell membranes.<sup>78</sup> Piao et al.<sup>79</sup> utilized a cationic liposome system for the delivery of pre-miR-107 in the treatment of head and neck squamous cell carcinoma. The miRNA-based treatment was delivered systemically in 8-week-old female athymic nude mice that



**Figure 4. A Venn Diagram Displaying let-7c Database Targets Using miRSearch 3, miRSeI, and starBase 2 Databases**

In total, four hsa-let-7c targets were identified as being common to all three databases.

had been implanted with human tongue squamous cell carcinoma (CAL27) cells. The systemic delivery of the miRNA-based treatment resulted in a significant ( $*p < 0.05$ ) stunt in tumor growth compared with the pre-miRNA-control group.<sup>79</sup> For cancer therapy, the ideal non-viral delivery system must be non-toxic and non-immunogenic, so as not to compromise healthy tissue in the process.

### Conclusions and Future Prospects

PCa is characterized by aberrant expression of several tumor suppressor or oncogenic miRNAs.<sup>47</sup> These miRNAs have the potential to serve as biomarkers for PCa identification,<sup>80</sup> as well as therapeutic targets for the treatment of PCa. MiRNA also has the potential to serve as a prognostic indicator in PCa.<sup>81</sup> This technology may be utilized in the era of personalized medicine for patient stratification and treatment.

Over the past number of years, miRNA expression has been profiled in a range of malignancies. Future experimental studies will shed light on the function and the contributory status of each miRNA in PCa pathogenesis. Each miRNA acts on and regulates many target genes. Aberrant and seemingly chaotic gene expression may be a result of dysregulation in only a very small number of miRNAs. Each aberrantly expressed miRNA presents a potential therapeutic target that can be used as a tool to normalize target gene expression. By normalizing gene expression, the expectation is that the cellular characteristics of malignancy can be halted or even reversed. Studies have shown that dysregulated miRNA expression leads to cellular proliferation, EMT, and therapeutic insensitivity, which can be abrogated.

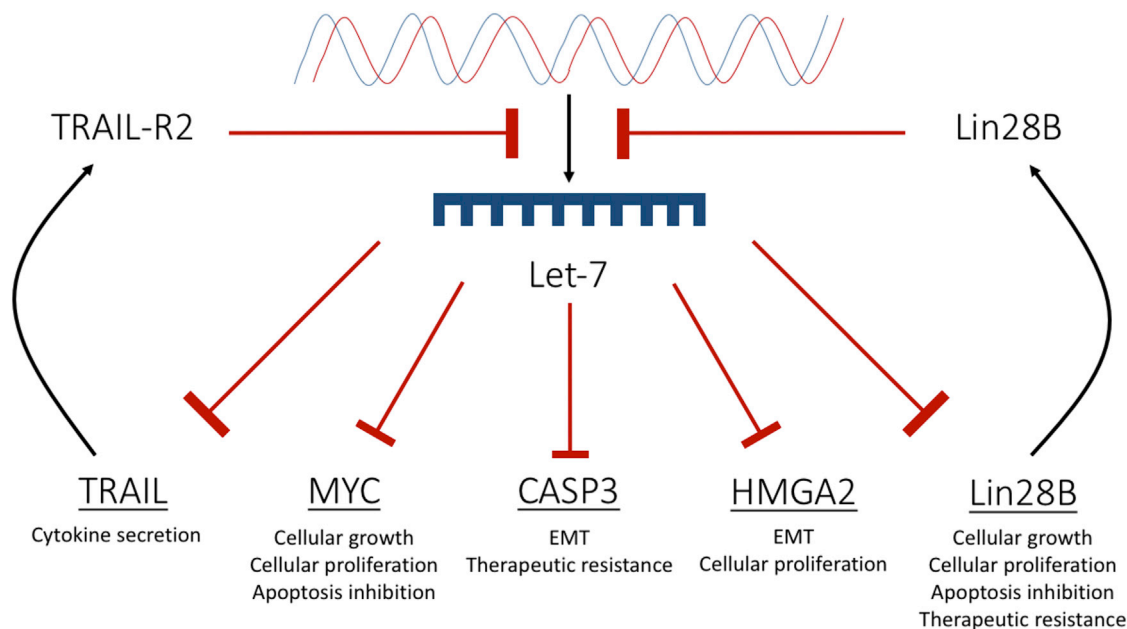
**Table 4. Hsa-let-7c Common Targets from Database Results**

Database Comparison	Target Genes Identified	Total
miRSearch 3.0 miRSeI starBase 2.0	HMGA2; MYC; TNFSF10; CASP3	4
miRSearch 3.0 miRSeI	BCL2; BCL2L1; TRIM71	3
miRSearch 3.0 starBase 2.0	VPS33A; PRTG; QARS; PXT1; BTN2A1; TAF9B; IGF2BP3; RNF170; C8orf58; CEP135; DDX19B; ABL2; YOD1; GDF6; DPH3; C14orf28; SMUG1; FAS; ZNF232; RICTOR; CLP1; COIL; DCUN1D3; ZCCHC9; IFT80; NRAS; ZNF275; ARHGAP28; CDK6; LIN28B; SUB1; ARPP19; SCD; ZNF792; MAP4K3; TBKBP1; TGFBR1; ZBTB26; ACVR1C; DNA2; E2F5; ADRB2; SOCS4; AHCTF1; PPAPDC1B; POLQ; MAP3K1; ABT1; PARS2; MBD2; NPHP3; DOT1L; PEX11B; POLR2D; CCR7; NAP1L1; USP38; CLDN12; STX3; DDX19A; FAM103A1; DVL3; DUSP3; MAPK6; RNFT1; CD80; SENP2; SFT2D3; CCNJ; HAND1; DOK3; RRM2; EIF3J; ABCC5; C15orf39; ZFYVE26; XRN1; ERCC6; ARID3B; MRS2; CDV3; SEMA4F; GJC1; CYB561D1; CDC34; USP12; C9orf40; CTPS2; PCTP; BRF2; TMEM2; TNFSF9; KCTD21; GALNT1; BCAT1; PBX2; NEK3; RGPDP6; C18orf21; PDE12; LIPT2; CDC25A; KHNYN	103
miRSeI starBase 2.0	MECP2; DHDDS; VIM; CDK4; PPARA	5

This review has identified members of the let-7 family, in particular, let-7c, as possible therapeutic targets in PCa. It was concluded that let-7c is typically downregulated in PCa, and targets include HMGA2, MYC, TNFSF10 (TRAIL), and CASP3. Therefore, let-7c suppression may lead to enhanced metastatic capabilities and a heightened stem-like tumor cell population.

Rather than attempting to highlight and treat each blockage, let-7c replacement therapy circumvents the obstruction and artificially restores let-7c to pre-malignant levels. This treatment aims to restore functionality to the cell rather than triggering cell death. This is, of course, an ideal scenario, where artificial replacement or suppression of miRNAs results in a restoration of functionality. In reality, however, it is more plausible that this therapy is used in conjunction with already established therapies to improve sensitivity.

Conventional therapeutics such as chemotherapy and radiotherapy aim to create intolerable endogenous levels of reactive oxygen species within the malignant cells in an attempt to trigger apoptosis. Surgical resection, meanwhile, seeks to remove the affected tissue to prohibit further growth. Personalized miRNA therapy aims to treat cancer from a new standpoint. By using miRNA replacement or antagomiRNAs, the goal is to reset cellular miRNA levels. The exciting prospect for this therapy is that the initial blockage that triggered the miRNA dysregulation is bypassed allowing levels to be restored artificially. RNA therapeutics are gaining much



**Figure 5. A Schematic Diagram Showing Targets of let-7 in Cancer**

Let-7 causes translational suppression of HMGA2, MYC, TRAIL, CASP3, and Lin28B. Blockage of let-7 biogenesis will consequently result in an upregulation of each of these targets. Lin28B and TRAIL-receptor 2 further contribute to the inhibition of let-7 biogenesis. Collectively, upregulation of these targets results in a proliferative, invasive, and therapeutically resistant phenotype.

momentum. For example, Onpattro, an RNAi therapy developed by Alnylam Pharmaceuticals for the treatment of adult patients with polyneuropathy of hereditary transthyretin-mediated amyloidosis, is the first of its kind. Onpattro contains a small interfering RNA (siRNA) formulated with a lipid complex and is termed patisiran. The drug works by binding to the TTR protein, thus preventing deformation through the RNAi. The reduction in TTR protein levels in the liver results in a decrease in subsequent amyloid deposits. Onpattro was approved by the FDA (August 2018) based on the highly positive outcome of a global phase III clinical trial. This trial was termed APOLLO and was a placebo-controlled, randomized, double-blind evaluation of the efficacy and safety of Onpattro in patients. In total, 225 patients were enrolled in APOLLO and treated over the course of 18 months. Within the trial 148 patients received Onpattro once every 3 weeks (0.3 mg/kg body weight), whereas the remaining received the placebo. Patients receiving the therapy showed improvements, with 51% of patients exhibiting an improved quality of life, as measured by the Norfolk Quality of Life Diabetic Neuropathy (QoL-DN), as opposed to only 10% in the placebo control.<sup>82</sup>

Of course, the efficacy of any miRNA therapy is reliant on an effective delivery system. This system must be designed in a way that would overcome both extracellular and intracellular obstacles, ensuring the successful shipment of the cargo resulting in the desired effect without eliciting an immune response. It would also be beneficial for such a vector to be specific to the site of action as not to elicit any off-target effects elsewhere in the body.

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