Table 1 5-Year incidence rates of distant recurrence

Treatment	Risk classification	Probability (95% CI)	Adjuvant therapy	1–5 Years (95% CI)	6–10 Years (95% CI)	Source
St. Gallen criteria-guided	High	0.8984 (0.8643 to 0.9325)	Chemotherapy	0.1095 (0.0723 to 0.1467)	0.0779 (0.0440 to 0.1117)	[5–7, 10]
	Low	0.1016 (0.0675 to 0.1357)	Endocrine therapy alone	0.0323 (−0.0336 to 0.0981)	0.1000 (−0.0139 to 0.2139)	
The 70-gene prognosis-signature-guided	High	0.4623 (0.4060 to 0.5186)	Chemotherapy	0.1773 (0.1135 to 0.2411)	0.1035 (0.0472 to 0.1597)	
	Low	0.5377 (0.4814 to 0.5940)	Endocrine therapy alone	0.0366 (0.0075 to 0.0656)	0.0633 (0.0249 to 0.1017)	

CI confidence interval

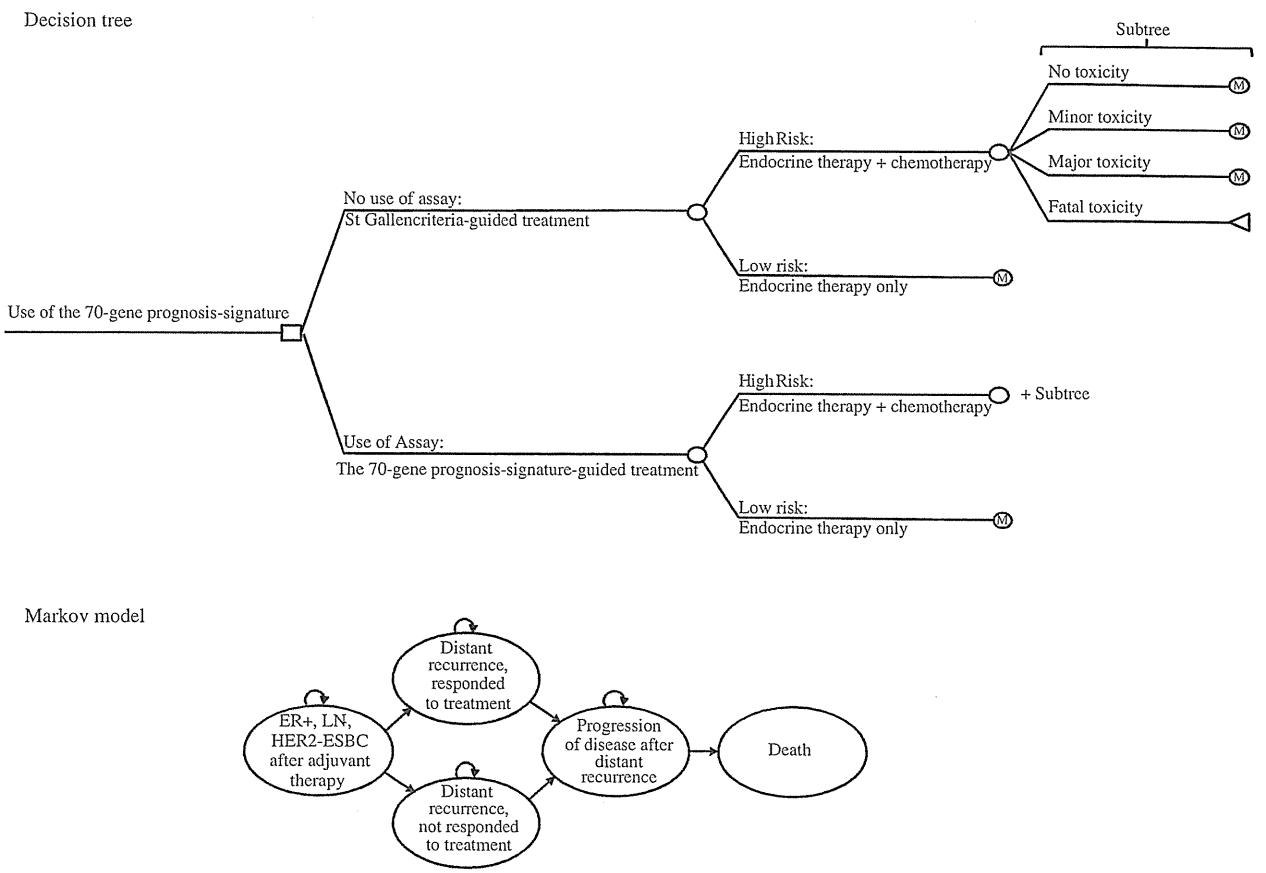

Fig. 1 Decision tree and Markov model

which is adopted and modified from our past studies [12, 13].

The decision tree corresponds to the comparison between St Gallen 2009 criteria-guided treatment versus the 70-gene prognosis-signature-guided treatment. The decision node of the tree is a decision whether to use the assay or not. Following chance nodes portion out the cohort to different adjuvant therapies depending on the risk classification. Here, we consider two types of adjuvant therapies: endocrine therapy plus chemotherapy for patients classified as at high risk of recurrence, and endocrine therapy alone for patients classified as at low risk of recurrence. Branches following 'plus chemotherapy' lead to subtrees via chance nodes, which portion out the cohort to different toxicities.

The Markov model shows the clinical course followed after the completion of adjuvant therapy. Five stages are modelled here: (1) ER+, LN−, HER2− ESBC after adjuvant therapy, (2) distant recurrence responded to treatment, (3) distant recurrence not responded to

treatment, (4) progression of disease after distant recurrence, and (5) death. Transitions between stages are indicated with arrows. Patients follow various courses after recurrence, and situations other than these five stages and transitions described here may be possible. However, we model the course this way based on the available reports of prognosis model of metastatic breast cancer, which is calibrated with the results of several randomised trials [12, 13, 16, 17]. So here, patients with recurrence undergo drug treatment with endocrine therapy or/and chemotherapy depending on their status.

The span of each stage is set at 1 year. Markov process is repeated up to 10 years, since the transition probabilities of recurrence are calculated by the 5-year incident rates of distant recurrence up to 10 years, and most of the recurrences are known to occur within this time horizon. After 10 years, a patient survived with no recurrence are assumed to have a life expectancy of 65-year-old Japanese female population [12, 13, 18], and those with recurrence are assumed to have a life expectancy of 2 years [12, 13, 19].

Outcomes estimation

Outcomes of each scenario in terms of life years (LYs) and quality adjusted life years (QALYs) are estimated by assigning probabilities and utility weights to the decision tree and Markov model from the literature.

Probabilities of risk classification, attached to the first chance node, are adopted from the results of a pooled prognosis analysis of three validation studies [5–7, 10] shown in Table 1. Table 2 shows other probabilities, utility weights, and costs used. Probabilities of adjuvant chemotherapy toxicity, which are attached to the chance node in the subtree, are assumed to be 60% for minor toxicity, 5% for major toxicity, and 0.5% for fatal toxicity according to the report of efficacy and cost-effectiveness of adjuvant chemotherapy in breast cancer [12, 13, 20].

In regards to the Markov model, transition probabilities of recurrence are calculated from the 5-year incident rates of distant recurrence depending on patients' status in Table 1. As mentioned above, transition probabilities between stages after recurrence are adopted from the prognosis model of metastatic breast cancer [12, 13, 16, 17]. Probabilities of the response to treatment for recurrence are fixed at 38.0% [12, 13, 17]. Probabilities of the progression of disease after recurrence are also fixed at: 59.7% if responded to the treatment and 98.3% if not responded to the treatment [12, 13, 16]. Probabilities of death after the progression of disease are fixed at 40.0% [12, 13, 16].

In order to estimate the outcomes in terms of QALYs, utility weights are chosen for various health states during the clinical course that patients follow. A weight for health states after adjuvant therapy without any toxicity or distant recurrence is chosen to be 0.98 [12, 13, 21]. Weights for toxicities are 0.90 for minor toxicity, and 0.80 for major toxicity [12, 13, 20], of which duration is assumed at 6 months. The health states during chemotherapy in preventing distant recurrence or the progression of disease weighs 0.50 [12, 13, 22], of which duration is assumed at 6 months. Health states after chemotherapy weigh 0.84 if responded to the treatment, 0.70 if stable, and 0.49 if the disease progressed [12, 13, 17].

Outcomes are discounted at a rate of 3% [23].

Costing

From the societal perspective, costing should cover the opportunity cost borne by various economic entities in the society. In the context of this study, costs borne by social insurers and patients are considered, since these two entities are the major payers to health care providers in Japan's social health insurance system. The amount of direct payments by these entities, according to the national medical care fee schedule, is estimated as costs, while costs of sector other than health and productivity losses are left uncounted in this study.

Cost items are identified along the decision trees and Markov model: the assay, adjuvant therapies, treatments for toxicity, monitoring, treatments for distant recurrence, and end-of-life treatments as shown in Table 2. The cost of the assay is ¥380,000 (US$4,222) according to the price offered by the Japanese supplier of MammaPrint®. Costs of treatments except the end-of-life treatments are estimated by combining a model of breast cancer care and the national medical care fee schedule. The care model is developed based on both a nationwide survey of Japanese expert practice and the consensus guidelines [12–15, 24].

Adjuvant endocrine therapy includes outpatient care with tamoxifen, aromatase inhibitors, and LH–RH analogues depending on patient's status, and it is assumed to continue up to 5 years, which costs ¥534,610/year (US$5,940/year) [12, 13]. Adjuvant chemotherapy includes various regimens. Anthracycline-based combination chemotherapy is used in about a half of all cases, and oral fluorinated pyrimidine and CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) therapy are frequently used among other regimens. These cost ¥343,001/year (US$3,811/year) [12, 13].

There are three levels of toxicity in the decision tree. However, only the cost of major toxicity is estimated as

Table 2 Probabilities, life expectancies, utility weights, and costs

	Base case value	Source
Probabilities		
Adjuvant chemotherapy toxicity		
Minor	60.0%	[12, 13, 20]
Major	5.0%	
Fatal	0.5%	
Responded to treatment for distant recurrence	38.0%	[12, 13, 17]
Progression of disease after distant recurrence		
Responded to treatment	59.7%	[12, 13, 16]
Not responded to treatment	98.3%	
Death after progression of disease	40.0%	[12, 13, 16]
Life expectancy at 10 year		
No distant recurrence	12.3	[12, 13, 18]
Distant recurrence	2.0	[12, 13, 19]
Utility weights		
After adjuvant therapy with no distant recurrence	0.98	[12, 13, 21]
Toxicity		
Minor	0.90	[12, 13, 20]
Major	0.80	
Distant recurrence		
Chemotherapy, 6 months only	0.50	[12, 13, 21]
If respond to treatment	0.84	[12, 13, 17]
Stable	0.70	
Progression of disease	0.49	
Costs		
The 70-gene prognosis-signature (MammaPrint®)	¥380,000	Local supplier
Adjuvant therapy		
Endocrine therapy (per year)	¥534,610	[12, 13]
Chemotherapy	¥343,001	
Treatment for toxicity		
Major	¥173,352	[12, 13, 25, 26]
Monitoring		
After adjuvant therapy with no recurrence (per year)	¥25,340	[12, 13]
Treatment for distant recurrence		
Endocrine therapy and chemotherapy (per year)	¥558,458	[12–15, 24]
End-of-life (per year)	¥1,315,143	[12, 13, 27]

¥173,352 (US$1,926). This includes an unplanned hospitalisation for 1 month in two-fifths of the cases, and rescue treatment at outpatient clinic in three-fifths of the cases [12, 13, 25, 26]. For minor toxicity, from which 60% of patients suffer, the cost is included in the cost of adjuvant chemotherapy, since prophylactic use of antiemetic, for example, is routinely applied these days. And the clinical course of fatal toxicity is so diverse and not fit to costing by the modelling here, therefore, its cost is assumed to be the same as the end-of-life treatments cited from the literature [12, 13, 27].

After the completion of adjuvant therapy, patients are assumed to visit their physician twice a year for the purpose of monitoring, of which cost is ¥25,340/year (US$282/year) [12, 13].

There are various options of treatments for distant recurrence depending on regimens used in the adjuvant therapy. Yet, we assume crossover hormonal treatments followed by capecitabine within the first year as a typical first line and second line therapies for our hypothetical cohort, which cost ¥558,458/year (US$6,205/year) [12–15, 24]. We further assume that this cost is applicable to the second year and thereafter.

The cost of the end-of-life treatments are ¥1,315,143/year (US$14,613/year)[12, 13, 27], which is also used as the cost of treating fatal toxicity.

Table 3 Results of cost-effectiveness analysis

Outcomes	Treatment	Cost	Incremental cost	Effect	Incremental effect	Incremental cost-effectiveness ratio
LY	St. Gallen criteria-guided	¥3,793,824		18.60 LY		
	The 70-gene prognosis-signature-guided	¥4,025,209	¥231,385	18.65 LY	0.048 LY	¥4,820,813/LY
QALY	St. Gallen criteria-guided	¥3,793,824		17.96 QALY		
	The 70-gene prognosis-signature-guided	¥4,025,209	¥231,385	18.02 QALY	0.060 QALY	¥3,873,922/QALY

LY life year, *QALY* quality adjusted life year

Costs are also discounted at a rate of 3% [23].

Comparison

Incremental cost-effectiveness ratios (ICER) are calculated:

$$\mathrm{ICER} = \frac{\mathrm{Cost}_{\text{The 70-gene prognosis-signature-guided treatment}} - \mathrm{Cost}_{\text{St Gallen criteria-guided treatment}}}{\mathrm{Effect}_{\text{The 70-gene prognosis-signature-guided treatment}} - \mathrm{Effect}_{\text{St Gallen criteria-guided treatment}}}$$

Although there is no established threshold value to interpret the ICER in Japan, some suggest social willingness-to-pay for one QALY gain from an innovative medical intervention in Japan as ¥5,000,000/QALY (US$55,556/QALY) [28]. We refer to this value in judging the cost-effectiveness.

Sensitivity analysis

In order to appraise the stability of ICERs against assumptions made and uncertainty of adopted values of probabilities, utility weights, and costs in our economic model, one-way sensitivity analyses are performed. The age of cohort is changed to 45 and 65 years old. Probabilities of risk classification and the 5-year incidence rates of distant recurrence shown in Table 1 are changed by 95% confidence interval. Probabilities and life expectancies shown in Table 2 are changed by ±50%. Utility weights shown in Table 2 are changed by ±20%. And costs shown in Table 3 are changed by ±50%. Discount rate is also changed from 0 to 5%.

Results

Cost-effectiveness

Table 3 shows the result of the cost-effective analysis of the 70-gene prognosis-signature. The cost of the 70-gene prognosis-signature-guided treatment, ¥4,025,209 (US$44,725), exceeds that of St Gallen criteria-guided treatment, ¥3,793,824 (US$42,154), which results in a positive incremental cost of ¥231,385 (US$2,571). The effect in terms of LYs of the 70-gene prognosis-signature-guided treatment, 18.65 year, exceeds that of St Gallen criteria-guided treatment, 18.60 year, which results in a positive incremental effect of 0.048 year. The ICER is calculated as ¥4,820,813/LY (US$53,565/LY). Similarly, the effect in terms of QALYs of the 70-gene prognosis-signature-guided treatment, 18.02 QALY, exceeds that of St Gallen criteria-guided treatment, 17.96 QALY, which results in a positive incremental effect of 0.060 QALY. The ICER is calculated as ¥3,873,922/QALY (US$43,044/QALY). According to the suggested social willingness-to-pay for one QALY gain, ¥5,000,000/QALY (US$55,556/QALY) [28], this is judged as cost-effective.

Stability of ICER

Table 4 shows the results of one-way sensitivity analyses.

The ICER is found very sensitive to clinical evidence depicting the treatment decision changes and the following 5-year incident rates of recurrence. Negative gains in outcomes are found in: increasing the probability of high risk guided by the 70-gene prognosis-signature; decreasing the probability of low risk guided by the 70-gene prognosis-signature; decreasing the 5-year incident rates after the St Gallen criteria-guided treatment; and increasing the 5-year incident rates after the 70-gene prognosis-signature-guided treatment. Cost-ineffective ICERs are found in: decreasing the probability of high risk guided by the St Gallen criteria; increasing the probability of low risk guided by the St Gallen criteria; and decreasing the 5-year incident rate from 1 to 5 year after the St Gallen crieteria-guided treatment for low-risk patients.

Table 4 Results of sensitivity analysis

	Range tested in sensitivity analyses	Incremental cost-effectiveness ratio (¥/QALY)	
		− Change	+ Change
Probabilities of risk classification			
St Gallen criteria-guided, high	Change by 95% CI	15,696,389	1,974,969
St Gallen criteria-guided, low		1,974,969	15,696,389
The 70-gene prognosis-signature-guided, high		729,324	Cost more, gain less
The 70-gene prognosis-signature-guided, low		Cost more, gain less	729,324
5-Year incidence rate of distant recurrence			
St Gallen criteria-guided, high, 1–5 years		Cost more, gain less	74,972
St Gallen criteria-guided, high, 6–10 years		Cost more, gain less	635,546
St Gallen criteria-guided, low, 1-5 years		147,550,296	1,968,870
St Gallen criteria-guided, low, 6–10 years		Cost more, gain less	1,920,488
The 70-gene prognosis-signature-guided, high, 1–5 years		123,080	Cost more, gain less
The 70-gene prognosis-signature-guided, high, 6–10 years		811,354	Cost more, gain less
The 70-gene prognosis-signature-guided, low, 1–5 years		588,308	Cost more, gain less
The 70-gene prognosis-signature-guided, low, 6–10 years		842,462	Cost more, gain less
Probabilities and life expectancies			
Adjuvant chemotherapy toxicity			
Minor	Change by ±50%	4,244,799	3,562,494
Major		3,970,536	3,780,250
Fatal		5,947,033	2,884,531
Responded to treatment for distant recurrence		3,873,334	3,874,347
Progression of disease after distant recurrence			
Responded to treatment		3,870,181	3,873,468
Not responded to treatment		3,873,493	3,873,832
Death after progression of disease		3,857,505	3,874,406
Life expectancy at 10 year			
No distant recurrence		5,211,728	3,084,132
Distant recurrence		3,868,265	3,879,420
Utility weights			
After adjuvant therapy with no distant recurrence	Change by ±20%	10,288,306	2,386,140
Toxicity			
Minor		2,780,389	6,384,768
Major		3,764,178	3,990,067
Distant recurrence			
Chemotherapy, 6 months only		3,873,184	3,875,130
If responded to treatment		3,873,849	3,873,498
Stable		3,873,498	3,873,849
Progression of disease		3,871,240	3,876,428
Costs			
The 70-gene prognosis-signature (MammaPrint®)	Change by ±50%	700,218	7,047,447
Adjuvant therapy			
Endocrine therapy (per year)		3,864,105	3,883,576
Chemotherapy		5,116,591	2,631,073
Treatment for toxicity			
Major		3,905,391	3,842,274
Monitoring			
After adjuvant therapy without recurrence (per year)		3,868,877	3,878,788
Treatment for distant recurrence			
Endocrine therapy and chemotherapy (per year)		3,876,226	3,871,438
End-of-life (per year)		3,875,557	3,872,125
Other assumptions			
Discount rate	0%/5%	2,606,613	4,448,622
Age of cohort	45/65 years old	3,456,614	4,536,315

QALY quality adjusted life year, *CI* confidence interval

The ICER is found relatively insensitive to probabilities, life expectancies, utility weights, costs, and other assumptions. However, cost-ineffective ICERs are found in: decreasing the utility weight after adjuvant therapy with no distant recurrence; increasing the cost of the 70-gene prognosis-signature; increasing the utility weight for minor toxicity; decreasing the probability of fatal toxicity; decreasing the life expectancy at 10 year with no recurrence; and decreasing the cost of adjuvant chemotherapy.

Discussion

We evaluate the cost-effectiveness of introducing the 70-gene prognosis-signature into Japanese practice of ER+, LN−, HER2− ESBC treatment. Our economic model indicates that the use of the signature gains more in terms of outcomes but costs more at the same time. The estimated ICER, ¥3,873,922/QALY (US$43,044/QALY) is not more than a suggested social willingness-to-pay for one QALY gain from an innovative medical intervention in Japan, ¥5,000,000/QALY (US$55,556/QALY) [29]. However, our sensitivity analysis shows the instability of this estimation as well. Changing the value of some variables results in negative gains in outcomes, or produce ICERs that is above the threshold. Therefore, we conclude that the introduction of the 70-gene prognosis-signature into Japanese practice of ER+, LN−, HER2− ESBC treatment has a reasonable, but not riskless chance to be judged as cost-effective and justified as an efficient deployment of finite health care resources.

In the sensitivity analysis, the prognosis prediction capacity of the assay is found most influential. This is plausible from the viewpoint of model construction. The range tested in regards to these variables is 95% confidence interval of the base-case values. So for this assumption, a larger patient pool of validation studies would reduce the instability. The costs of the assay and adjuvant chemotherapy are also found influential, which are as anticipated. Relative costs of these are a key factor for economic implication of the assay.

Since the Markov model used in this study is similar to our economic evaluation of another gene signature, the 21-gene signature, for similar patient population [12], a straightforward comparison can be made between the results. While the 21-gene signature predicts the benefits from chemotherapy in addition to the prognosis, which is modelled in our previous evaluation, this model is comparable in a way that we assume the predictable benefits of chemotherapy of the 70-gene prognosis-signature is zero. Regarding ER+, LN−, HER2− diseases, the introduction of the 21-gene signature has more favourable ICER, ¥434,096/QALY (US$4,823/QALY), than the results of this study. However, due caution is needed to interpret this comparison because the breadth of indication for other patient population or other setting such as the prediction of response to neoadjuvant therapy is different from each other, which inevitably affect the value for money of the assay on every count. And the differences in clinical validation studies of these gene signatures make the comparison profoundly complicated. For example, the difference of simplified patient characteristics in each economic model may have a substantial relevance. The choice of clinical endpoint in the economic modelling, such as between local recurrence response and overall survival, may also be significant.

Although no direct comparison can be made between economic evaluations conducted under different health systems [29, 30], the cost-effectiveness of the 70-gene prognosis-signature for ESBC patients found in this study is consistent with the findings of past reports from The Netherlands [10] and the US [11], which found the use of assay cost-effective in each context.

This study has its own limitations. First of all, the clinical evidence depicting the treatment decision change and prognosis to recurrence is adopted from a pooled study of validation studies overseas. Its representativeness of Japanese patient population targeted in this study is inevitably questionable and racial differences should exist. Although we justify our approach taken as the best available evidence to date, further analyses based on Japanese clinical data are awaited. Our previously conducted economic evaluations of the 21-gene signature were analysed in two phases: early analysis using clinical evidence overseas [13] and late analysis using data from Japanese validation study [12]. This experience suggests that there is a room for different results as to the 70-gene prognosis-signature as well. Second, the quotation of an established economic model of courses followed by the target patients [12, 13] may fail to catch up with the latest developments in breast cancer treatments. For example, our Markov model assumes the so-called second generation adjuvant chemotherapies. But the use of third generation adjuvant chemotherapies is still limited in Japan [31], and no remarkable change has been made about adjuvant endocrine therapies in the Japanese consensus guideline [15] since our previous study. And therefore, we think that the quotation from the past model is still acceptable for the purpose of this study. Third, utility weights adopted are also derived from western countries due to the unavailability of data from Japan. Fourth, due to the same reason, our model does not include potentially costly clinical stages such as local recurrence or contralateral breast cancer. In regards to these shortcomings, reports that allow us to refine our model are awaited.

In considering the routine use of expensive biomarkers such as gene signatures, the appraisal of cost-effectiveness is imperative [32] with growing concerns globally about financing medical advancements [33]. The results of this study imply that the diffusion of the assay is potentially acceptable under Japan's health system from the viewpoint of health economics.

However, there is also a concern about the novelty of such biomarkers under severe health care resources constraints. Biomarkers for individualised treatments imply more 'cost-saving' by avoiding unnecessary care than expensive new drugs, while its approval process is often different from pharmaceuticals. Some health managers in Japan and elsewhere may intuitively think their routine use is financially acceptable only when 'cost-saving' results are reported in economic evaluations. However, from the viewpoint of economic evaluation, it is not justifiable to set different thresholds between biomarkers and pharmaceuticals. For example, an expensive drug therapy, adjuvant trastuzumab treatment, is included in Japan's social health insurance benefit package, although it has been found cost-effective but not cost-saving [34]. Exploration of financing strategy beyond the conventional cost-effectiveness analysis may be needed.

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Conflict of interest All authors declare that there is no possible conflict of interest.

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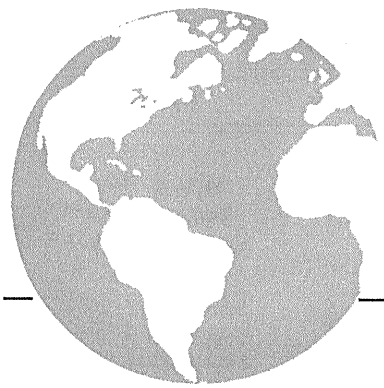

MicroRNA let-7: an emerging next-generation cancer therapeutic

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ABSTRACT

In recent years, various RNA-based technologies have been under evaluation as potential next-generation cancer therapeutics. MicroRNAs (miRNAs), known to regulate the cell cycle and development, are deregulated in various cancers. Thus, they might serve as good targets or candidates in an exploration of anticancer therapeutics. One attractive candidate for this purpose is let-7 ("lethal-7").

Let-7 is underexpressed in various cancers, and restoration of its normal expression is found to inhibit cancer growth by targeting various oncogenes and inhibiting key regulators of several mitogenic pathways. *In vivo,* let-7 administration was found effective against mouse-model lung and breast cancers, and our computational prediction supports the possible effectiveness of let-7 in estrogen receptor (ER)–positive metastatic breast cancer. Data also suggest that let-7 regulates apoptosis and cancer stem cell (CSC) differentiation and can therefore be tested as a potential therapeutic in cancer treatment. However, the exact role of let-7 in cancer is not yet fully understood. There is a need to understand the causative molecular basis of let-7 alterations in cancer and to develop proper delivery systems before proceeding to therapeutic applications. This article attempts to highlight certain critical aspects of let-7's therapeutic potential in cancer.

KEY WORDS

Let-7, microRNA, cancer therapy, let-7 regulation, future medicine

1. INTRODUCTION

MicroRNAs (miRNAs) are natural non-coding RNAs of approximately 22 nucleotides (nt) in size. They regulate genes post-transcriptionally by binding to a site in the 3′ untranslated region (UTR) of target messenger RNAs (mRNAs). Identification of an miRNA target involves base pairing with the target site, which is mostly imperfect in the case of animals. However, a perfect pairing in a 7-nt region at the 5′ end of miRNA, called the seed region, is essential for target identification [1].

The miRNAs are known to regulate cellular processes such as stem-cell differentiation, heart development [2–4], insulin secretion [5], apoptosis [6,7], aging [8,9], and immunity [10,11], among other processes. It is therefore not surprising that miRNAs are differentially expressed in several pathophysiologic conditions including, for instance, Alzheimer disease [12,13], Parkinson disease [14], cardiovascular diseases [4,15,16], the Cowden and Down syndromes [17,18], and various cancers [19].

Let-7 was first discovered and well studied in *Caenorhabditis elegans,* in which it regulates developmental timing [20–23] (larval stage 4–to–adult transition [20,24]) and stage-specific neuromuscular tissue development [25]. Let-7 has orthologs in various species. In *Drosophila,* let-7 plays a role in determining the timing for cell-cycle exit, metamorphosis, neuromuscular Junction development, juvenile-to-adult-stage transition, and adult behaviour [26,27]. The zebrafish ortholog of let-7 is prominently expressed in nervous tissue, indicating its certain role in neural development [28]. In the adult newt, let-7 regulates transdifferentiation and regeneration of lens and inner ear-hair cells [29].

Little is known about the function of let-7 in mammalian development and normal physiology. In the mouse, let-7 is involved in neural lineage specificity of embryonic stem cells, brain development [30], and mammary epithelial progenitor cell maintenance by induction of loss of self-renewal [31]. In humans, 12 genomic loci encode the let-7 family members (let-7a-1, -2, -3; let-7b; let-7c; let-7d; let-7e; let-7f-1, -2; let-7g; let-7i; MIR98). Human let-7 is upregulated during embryonic cell differentiation [32], but the roles it plays in normal physiology are mostly unknown.

Human let-7 family members are found to be downregulated in several cancers, with a few exceptions (Table I); restoration of normal expression prevents tumorigenesis [37,44,45,52]. Let-7 therefore acts as a tumour suppressor and a regulator of terminal differentiation and apoptosis. This finding implies that let-7 can possibly be used as a next-generation cancer therapeutic. But, to date, the mechanism of let-7

TABLE I Deregulation of microRNA let-7 family members in various cancers

Cancers	*MicroRNA let-7 family members*	*References*
Cancers that exhibit downregulation of specific let-7 family members		
Acute lymphoblastic leukemia	let-7b	Mi *et al.*, 2007 [33]
Bladder cancer	let-7b, let-7d, let-7e, let-7f	Nam *et al.*, 2008 [34]
Breast cancer	let-7, let-7a	Sempere *et al.*, 2007 [35]
		Yu *et al.*, 2007 [36]
Bronchioloalveolar cancer	let-7	Inamura *et al.*, 2007 [37]
Burkitt lymphoma	let-7a	Sampson *et al.*, 2007 [38]
Colon cancer	let-7	Michael *et al.*, 2003 [39]
		Akao *et al.*, 2006 [40]
		Fang *et al.*, 2007 [41]
Gastric cancer	let-7	Zhang *et al.*, 2007 [42]
		Motoyama *et al.*, 2008 [43]
Hepatocellular cancer	let-7	Johnson *et al.*, 2007 [44]
Kidney cancer	let-7a, let-7c, let-7d, let-7e, let-7f, let-7g	Nam *et al.*, 2008 [34]
Lung cancer	let-7	Johnson *et al.*, 2007 [44]
		Takamizawa *et al.*, 2004 [45]
		Johnson *et al.*, 2005 [46]
Malignant melanoma	let-7b	Schultz *et al.*, 2008 [47]
Ovarian cancer	let-7a-3	Lu *et al.*, 2007 [48]
Pancreatic cancer	let-7	Jérôme *et al.*, 2007 [49]
Prostate cancer	let-7c	Jiang *et al.*, 2005 [50]
Cancers that exhibit upregulation of specific let-7 family members		
Acute myeloid leukemia	let-7	Garzon *et al.*, 2008 [51]
Breast cancer	let-7b	Nam *et al.*, 2008 [34]
Colon cancer	let-7a, let-7g	Nam *et al.*, 2008 [34]
Lung cancer	let-7a	Nam *et al.*, 2008 [34]
Retinoblastoma	let-7a, let-7b, let-7c	Nam *et al.*, 2008 [34]
Uterine cancer	let-7i	Nam *et al.*, 2008 [34]

deregulation, and its precise role in tumorigenesis, is not fully understood, creating a hurdle to effectively using this miRNA in cancer therapy.

This article presents an overview of let-7 and discusses the critical issues that must be explored to develop a let-7–based therapeutic strategy against various cancers.

2. DISCUSSION

2.1 Biogenesis and Mechanism of Action

The biogenesis of let-7 is similar to that of other miRNAs. The first step in miRNA biogenesis is transcription from the miRNA transcription unit by RNA polymerase II to produce a primary transcript called pri-miRNA. The pri-miRNA is processed by the microprocessor complex containing an RNase III–like enzyme, Drosha, and its co-factor, a double-stranded RNA binding protein, Dgcr8, to produce an approximately 60–70 nt pre-miRNA (precursor miRNA). The pre-miRNA is then transported to cytoplasm by exportin 5 (*XPO5*), in a RanGTP (*ras*-related nuclear protein–guanosine triphosphate complex)–dependent way, where it is cleaved by Dicer (a cytoplasmic RNase III), to generate an imperfect miRNA:miRNA* duplex of approximately 21–24 nt. One of the strands (the "guide strand") from the duplex is then incorporated into Argonaute (Ago)–containing ribonucleoprotein (RNP) complex; the other strand (the "passenger strand") is degraded. However, there are cases in which both strands of the duplex are detected in the cell [53]. The miRNA–Ago RNP complex causes posttranscriptional regulation of genes, in which miRNA is used as a tether to guide the complex to the specific mRNA. The exact mechanism by which the miRNP complex regulates expression of the target remains unclear. Various models try to explain this mechanism [1]. Figure 1 shows a general model.

2.2 Regulation of Let-7

Expression of let-7 is regulated at various stages of its biogenesis and also depending on cell type. Similarly,

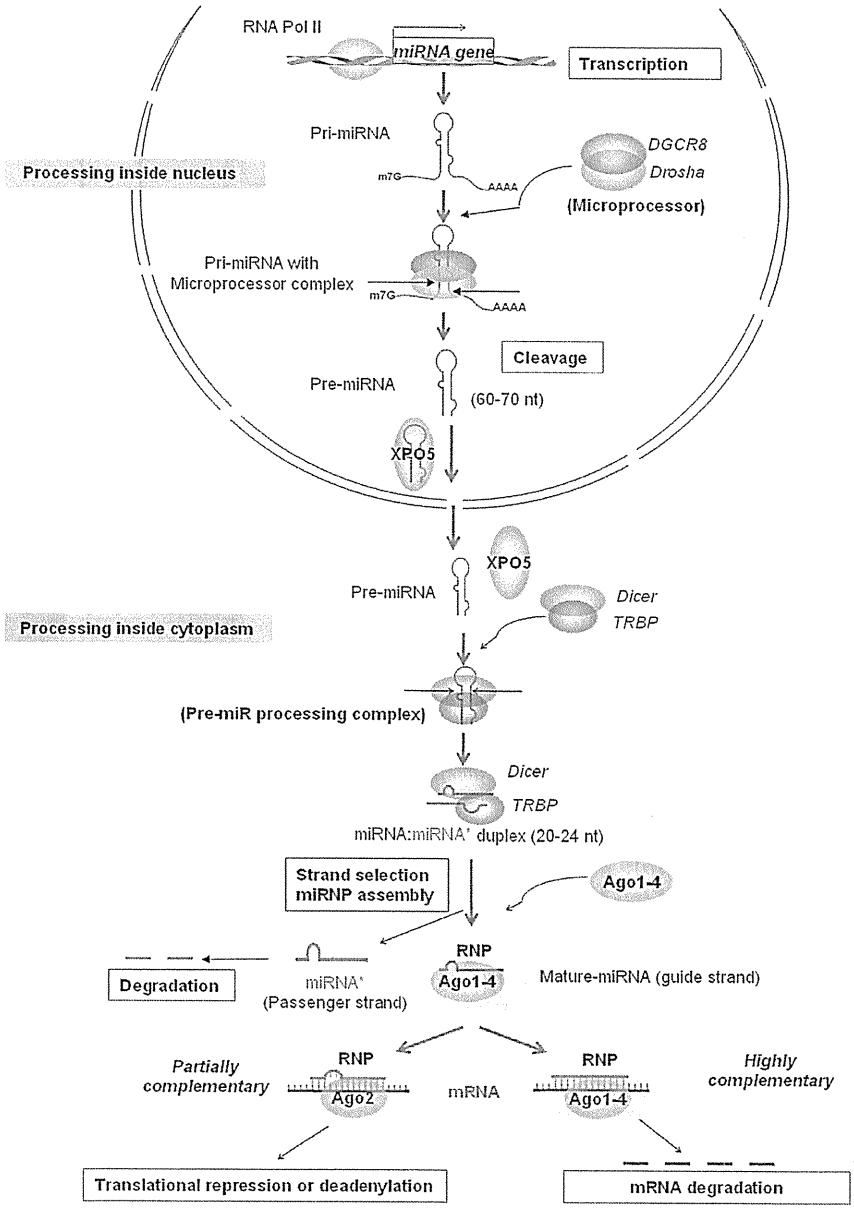

FIGURE 1 *The most-accepted model of microRNA (miRNA) biogenesis and its mechanism of action. For detail, see text. RNA Pol II = RNA polymerase II; Pri-miRNA = primary transcripts of miRNA; DGCR8 = DiGeorge syndrome critical region gene 8; Drosha = class 2 RNASE III enzyme; XPO5 = exportin 5; Dicer = formal symbol DICER1 (dicer 1, ribonuclease type III); TRBP = now labelled TARBP2P [TAR (HIV-1) RNA binding protein 2 pseudogene]; Ago1–4 = Argonaute-1 to -4 [symbol EIF2C1, 2, 3, 4 (eukaryotic translation initiation factor 2C, 1–4)]; RNP = ribonucleoprotein; mRNA = messenger RNA.*

let-7 regulates many transcription factors that play important roles in regulation of the cell cycle, cell differentiation, and apoptosis. Many of the factors controlling the expression of let-7 form regulatory circuits with the factors being regulated by such expression. These regulatory circuits—such as double-negative feedback loops and so on—are salient network motifs in development and differentiation. *LIN28, POU5F1, SOX2, NANOG, TLX1, HMGA2, MYC,* and *IMP*s are known to form such regulatory loops (Figure 2).

2.2.1 Regulation of Let-7 by Pluripotency-Promoting Factors in Embryonic and Cancer Stem Cells

LIN28, which maintains the undifferentiated state of embryonic cells, is a well-known target of let-7 and is downregulated by let-7 during developmental

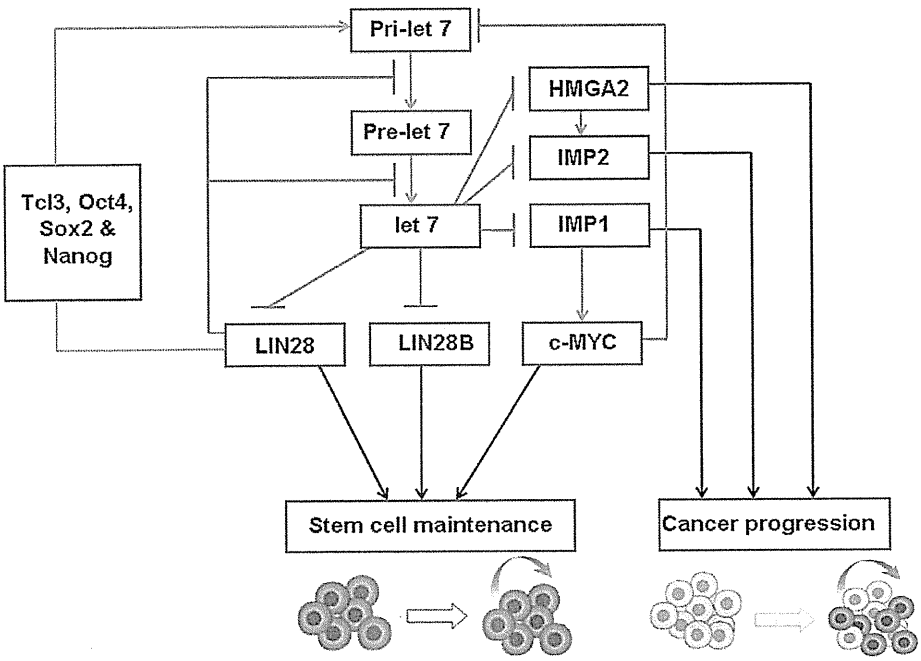

FIGURE 2 *Regulatory circuits of microRNA (miRNA) let-7. The loop consists of pluripotency promoting factors {LIN28 [lin-28 homolog* (Caenorhabditis elegans)*], OCT4 [now labelled POU5F1 (POU class 5 homeobox 1)], SOX2 [SRY (sex determining region Y)–box 2], NANOG [Nanog homeobox], and TCL3 [now labelled TLX1 (T-cell leukemia homeobox 1)]}, oncofetal genes [HMGA2 (high mobility group AT–hook 2) and IMPS (insulin-like growth factor 2 mRNA-binding proteins)], and oncogene MYC. For detail, see text. Pri-let 7 = primary transcripts of let-7; LIN28B = lin-28 homolog B* (C. elegans).

commitment [54,55]. Lin28 was recently shown to act as a posttranscriptional repressor of let-7 biogenesis, binding to the loop portion of the pri–let-7 hairpin and the stem part of pre–let-7 and thereby inhibiting its processing. Lin28 and Lin28B also inhibit processing of let-7 by mediating terminal uridylation of let-7 precursors [56]. What is unclear is whether the regulation by Lin28 occurs at the Drosha or Dicer processing step [55,57–59]. Lin28 induces pri–let-7 expression through induction of other pluripotency-promoting factors such as Pou5F1, Sox2, Nanog, and Tlx1 [60], thus regulating let-7 expression at multiple levels.

The early embryonic oncofetal gene *HMGA2* is involved in the self-renewal and maintenance of adult stem cells. It is highly expressed in hematopoietic and fetal neuronal stem cells [61,62], and the low levels of let-7 in stem cells inversely correlate with *HMGA2* expression. Thus, the undifferentiated state is maintained [63]. In differentiated tissues, *HMGA2* is downregulated because of the high expression of let-7 [61], and during induced differentiation, ectopic expression of let-7 reduces *ras* and *HMGA2* expression, leading to inhibition of cell proliferation and induction of apoptosis. Therefore, *HMGA2* is a direct target of let-7 [64].

Like normal stem cells, cancer stem cells (slowly dividing tumour-initiating cells) exhibit low levels of let-7 and possess unlimited self-renewal capability and pluripotency, allowing them to repopulate and metastasize [65,66]. It has been proposed that, during carcinogenesis, the let-7–targeted embryonic genes, which are otherwise not expressed in adult tissues, are re-expressed because of loss of let-7 control. This reprogramming promotes de-differentiation and cancer progression [67]. A good example is that of *HMGA2,* which is undetectable in most differentiated tissues, but highly expressed in various cancers, including neuroblastoma and pancreatic, lung, and thyroid cancers [68–71]. Breast cancer stem cells are also devoid of let-7, but abundantly express *HMGA2* and *ras* [36] (Figure 2).

2.2.2 Regulatory Circuit Between Myc and Let-7

IMP1 is another oncofetal gene that is expressed only during early fetal life [72,73] and is re-expressed in several cancers [74]. It is selectively expressed in young, but not in old, hematopoietic stem cells [75]. *IMP1* regulates stem cell functions by stabilizing insulin-like growth factor 2 and C-*myc* mRNAs [76,77], and the phenotype of stem cells from the *IMP1* knockout mouse resembles that of cells from the *HMGA2*-deficient mouse [73,78]. Let-7 targets *IMP1*, and therefore indirectly acts as a negative regulator of *MYC* expression [64,79,80]. It has been shown that Myc binds directly to let-7 promoter and downregulates its transcription [81]. Thus, an indirect feedback circuit exists between let-7 and Myc (Figure 2).

2.3 Let-7 Targets Multiple Oncogenes and Components of Cell Cycle, Cell Proliferation, and Apoptosis

Apart from targeting oncogenes (*ras, MYC, HMGA2*, and so on) as already discussed, let-7 regulates several key components of the cell cycle and cell proliferation. Microarray analysis of hepatocellular carcinoma (HepG2) and lung cancer (A549) cell lines revealed that let-7 inhibits multiple cell-cycle- and proliferation-associated genes, including cyclin A2 (*CCNA2*), *CDC34*, Aurora A [*AURKA* (formerly *STK6*)] and B [*AURKB* (formerly *SKT12*)] kinases, *E2F5, CDK8*, and *PLAGL2*, among others[46]. In HepG2 cells, let-7 directly represses *CCNA2, CDC25A, SKP2, AURKA, CDC16, CCND1*, and *CDK6*, among others. Let-7 also inhibits several DNA replication machinery components (*ORC1L; RRM1, 2*; and so on) and transcription factors [*E2F6, CBFB, PLAGL2, SOX9, GZF1* (formerly *ZNF336*), *YAP1, GTF2I, ARID3A*, and so on]. Surprisingly, that study also showed that let-7 represses several tumour suppressor genes (*BRCA1, BRCA2, FANCD2*, and *PLAGL1*, among others) and checkpoint regulators (*CHEK1, BUB1, BUB1B, MAD2L1*, and *CDC23*, among others). Our recent *in silico* analysis shows that let-7 may potentially target ER signalling and angiogenic pathways by targeting key molecules of these cascades[82]. Various targets of let-7 are listed in Table II and shown in Figure 3.

Apoptosis regulatory functions of let-7 have recently been reported in both human and mouse. Let-7 targets Casp3 in the A431 and HepG2 cell lines, and inhibits doxorubicin- and paclitaxel-induced apoptosis[85]. In NIH3T3 mouse fibroblast cells, let-7

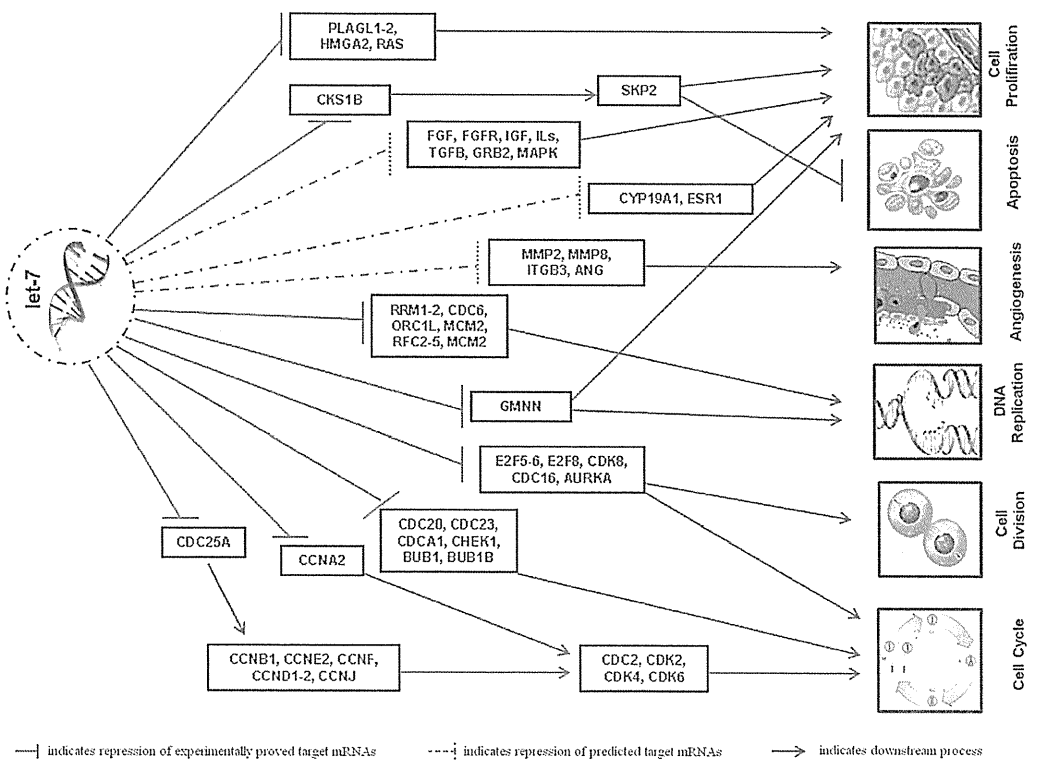

FIGURE 3 *Let-7 targets various key components of mitogenic and tumorigenic pathways to exert its tumour suppressor activity. Pathways include cell cycle, cell division, cell proliferation,* DNA *replication, angiogenesis, and apoptosis. PLAGL1, 2 = pleomorphic adenoma gene-like 1, 2; CKS1B =* CDC28 *protein kinase regulatory subunit 1B; SKP2 = S-phase kinase-associated protein 2 (p45);* FGF, FGFR *= fibroblast growth factor and fibroblast growth factor receptor;* IGF *= insulin-like growth factor;* IL-S *= interleukin S;* TGFB *= transforming growth factor β; GRB2 = growth factor receptor-bound protein 2;* MAPK *= mitogen-activated protein kinase; CYP19A1 = cytochrome P450, family 19, subfamily A, polypeptide 1; ESR1 = estrogen receptor 1; MMP2, 8 = matrix metallopeptidases 2, 8; ITGB3 = integrin β3; ANG = angiogenin; RRM1, 2 = ribonucleotide reductases M1 and M2; CDC6 = cell division cycle 6 homolog* (Saccharomyces cerevisiae)*; ORC1L = origin recognition complex, subunit 1-like (yeast); MCM2 = minichromosome maintenance complex component 2; RFC2–5 = replication factor C (activator 1) 2–5; GMNN = geminin,* DNA *replication inhibitor; E2F5, 6, 8 =* E2F *transcription factors 5, 6, 8; CDK8 = cyclin-dependent kinase 8; CDC16 = cell division cycle 16 homolog* (S. cerevisiae)*; AURKA = aurora kinase A; CDC25A = cell division cycle 25 homolog A* (Schizosaccharomyces pombe)*; CCNA2 = cyclin A2; CDC20, 23 = cell division cycle 20 and 23 homologs* (S. cerevisiae)*; CDCA1 = (now labelled NUF2) NDC80 kinetochore complex component, homolog* (S. cerevisiae)*; CHEK1 =* CHK1 *checkpoint homolog* (S. pombe)*; BUB1, 1B = budding uninhibited by benzimidazoles 1 and 1 β homologs (yeast); CCNB1, D1, D2, E2, F, J = cyclins B1, D1, D2, E2, F, J; CDC2 = cell division cycle 2, G1 to S and G2 to M; CDK2, 4, 6 = cyclin-dependent kinases 2, 4, 6;* MRNA *= messenger* RNA.

TABLE II MicroRNA let-7 targets in various cancers

Cancer	*MicroRNA let-7*			*Model used*	*References*
	Expression	*Targets*	*Effect on targets*		
Breast cancer	let-7 ↓	*ANG*; *CCND1, 2*; *CDC25A*; *CDK4, 6*;*CYP19A1*; DNA polymerases; *E2F5, 6*; *ESR1, 2*; *FGF11*; FGFR; *GRB2*; *HMGB2*; *IGF1, 1R*; *IL6*; *ITGB3*; *MAPK4, 6*; *MMP2*; *MMP8*; *MYC*; *ras*; *RB1*; *SKP2*; *TGFB1, BR1*; *TP53*	Transcription	*In silico*	Barh *et al.*, 2008 [82]
	let-7 ↓	*HMGA2*, H-*ras*	Transcription	Cell line, mouse model	Sempere *et al.*, 2007 [35] Yu *et al.*, 2h007 [36]
Burkitt lymphoma	let-7a ↓	*MYC*	Transcription/translation	Cell line	Sampson *et al.*, 2007 [38]
Colon cancer	let-7 ↓	*ras*, *MYC*	Translation	Cell line	Akao *et al.*, 2006 [40]
Hepatocellular cancer	let-7 ↓	*AURKA*; *BRCA1, 2*; *BUB1*; *CCNA2, B1, E2, F, J*; *CDC2, 6, 20, 23, 25A, 34, 45L*; *NUF2*; *CBX2*; *CDCA2, 3, 4, 5, 7, 8*; *CDK8*; *CHEK1*; *CKS1B*; *DBF4*; *DICER1*; *E2F5, 6, 8*;*FANCD2*; *GMNN*; *CDT1*; *HMGA2*; *LIN28B*; *MAD2L1*; *NRAS*; *ORC1L*; *PLAGL1, 2*; *RRM1, 2*; *SKP2*; *SOX9*; *ARUKB* (formerly STK12)	Transcription	Cell line	Johnson *et al.*, 2007 [44]
Lung cancer	let-7 ↓	*MYC*, *ras*	Transcription/translation	Cell line	Johnson *et al.*, 2005 [46] Kumar *et al.*, 2008 [52]
	let-7 ↓	*AURKA*; *CCNA2*; *CDC34*; *CDK8*; *DBF4*; *DICER1*; *E2F5*; *GMNN*; *HMGA2*; *LIN28B*; *NRAS*; *PLAGL1, 2*; *ARUKB* (formerly STK12)	Transcription	A549 lung cancer cells	Johnson *et al.*, 2007 [44]
	let-7 ↓	*HMGA2*	Transcription	Cell line	Kumar *et al.*, 2008 [52] Lee and Dutta, 2007 [83]
Malignant melanoma	let-7b ↓	*CDK4*; cyclins A, D1, D3	Translation	Cell line	Schultz *et al.*, 2008 [47]
Uterine leiomyoma	let-7 ↓	*HMGA2*	Transcription	Tumour sample, cell line	Peng *et al.*, 2008 [84]

FGFR = fibroblast growth factor receptor; ↓ = downregulation.

is involved in ultraviolet B–induced apoptosis by modulating Casp3, Bcl2, Map3k1, and Cdk5 [86].

2.4 Emerging Role of Let-7 in Cancer Diagnosis and Therapy

The facts discussed here indicate that let-7 acts as a tumour suppressor by targeting various oncogenes and key components of the cell cycle and developmental pathways. Most reports reveal that let-7 is frequently underexpressed (Table I) and that the chromosomal region of human let-7 is frequently deleted in many cancers [87]. Similarly, in more differentiated tumour cells, let-7 is expressed at higher levels, and its target oncogenes (*HMGA2* and *ras*) are downregulated. Thus, loss of let-7 expression is a marker for less differentiated cancer [88], and expression levels are also found to be effective prognostic markers in several cancers [40,46,88]. In lung cancer, reduced let-7 expression was also found to significantly correlate with shortened postoperative survival regardless of disease stage [45].

From the therapeutic viewpoint, let-7 is attractive molecule for preventing tumorigenesis and angiogenesis [89]; it is a potential therapeutic in several cancers that underexpress let-7. Let-7 replacement was found to inhibit anchorage-independent growth and cell-cycle progression in melanoma cells by repressing regulators of the cell cycle and cell proliferation such as cyclins A, D1, and D3 and *CDK4* [47]. Together with *TP53, ras* and *MYC* have been implicated as key oncogenes in lung cancer. The reduced expression of let-7 in lung cancer directly correlates with upregulation of oncogene *ras*; introduction of let-7 represses *ras* and *MYC* translation by targeting the related mRNAs [45,46]. In both lung and hepatocellular carcinomas, replacement or restoration of normal expression levels of let-7 inhibits cancer growth by repressing multiple cell-cycle and proliferation pathways, together with *ras* and *MYC* [37,44,45,52] (Table II). Intranasal let-7 administration was found effective in reducing tumour growth in a K-*ras* mutant mouse model of lung cancer [90]. Similarly, restoration of let-7 restrains the growth and proliferation of colon and hepatic cancers [40,80]. Transfection of let-7 in a Burkitt lymphoma cell line downregulates *MYC* and reverts *MYC*-induced cell growth [38]. Ectopic expression of let-7 inhibits cell proliferation by directly repressing the *HMGA2* oncogene in lung cancers [52,83] and uterine leiomyoma [84].

Induced expression of let-7 in breast cancer cells targets *HMGA2* and H-*ras* [36], and in a mouse model of breast cancer, exogenous let-7 delivery suppresses cell proliferation, mammosphere formation, and the population of undifferentiated cells by downregulating both of the foregoing oncogenes [35,36]. In our *in silico* analysis, we recently showed that, apart from repressing *MYC, ras,* and *HMGA2,* let-7 may also target *CYP19A1, ESR1,* and *ESR2,* thereby potentially blocking estrogen signalling in ER-positive breast cancers. Similarly, by repressing angiogenin, fibroblast growth factor, transforming growth factor, interleukin 6, and matrix metallopeptidase 2, let-7 may prevent growth, angiogenesis, and metastasis in breast cancer [82] (Table II).

2.5 Limitations of Let-7–Based Therapy

2.5.1 Limitations Because of Limited Knowledge of Let-7 Biology

Although restoration of normal let-7 expression proves beneficial, limited knowledge concerning its transcriptional and processing control during biogenesis and its exact role in tumorigenesis make it difficult to directly apply let-7 as a therapeutic. It is necessary to know whether downregulation of let-7 in tumours is a primary or secondary phenomenon during tumorigenesis. Supporting the CSC hypothesis, we agree with the opinion that epigenetic downregulation of let-7 in CSCs leads to upregulation of oncofetal genes (*HMGA2* and *LIN28,* among others) and, thereby, to loss of differentiation and tumorigenesis. In that scenario, downregulation of let-7 is the primary event, a view that can be supported by observation of where in ovarian cancer let-7 is hypermethylated [48].

Because miRNAs act on the 3′ UTR of target mRNAs, it is important to determine how efficiently let-7 will work as a therapeutic, because 3′ UTR truncated oncogenes may be prevalent in neoplasia. Grimm *et al.* [91] reported that delivery of adeno-associated virus (AAV)–mediated recombinant pre-miRNAs causes death in mice from severe liver cytotoxicity. Details of the immunogenic and cytotoxic effects of let-7 therefore need to be explored so that such side effects can be minimized in an effective treatment strategy. Similarly, we proposed that let-7 may be involved in an as-yet-unknown regulatory network of miRNAs that resembles the gene regulatory network involving transcription factors. Therefore, anti-miRNA oligo-based knockdown of let-7 inhibitory miRNAs is not currently possible.

2.5.2 Limitations in Delivery Methods and Systems

Lack of an appropriate, safe, and effective delivery method for let-7 is another drawback of possible therapy. Biological vectors such as AAV and lentivirus may be used for targeted delivery [92], but standardization of the method is required to prevent non-targeted site introduction. Also, brain-specific miRNA delivery is not yet successful [93], and effective neuron-specific delivery methods have to be developed to tackle brain and neuronal tumours. As discussed earlier, AAV- and lentivirus-mediated delivery of let-7 in a mouse model of lung cancer [52,90] was found to be inefficient in pre-existing tumours because of the resistance to let-7 developed by the tumour over time [52]. A strategy for let-7–mediated therapy for pre-existing tumours therefore also has to be developed.

2.6 Strategies to Overcome the Limitations

The optimal or normal level of let-7 may be restored in cancer cells either by administering exogenous

let-7 *in situ* with a vector overexpressing let-7, or by repressing let-7 repressors. Recent miRNA technologies are, in general, designed to use complementary or chemically modified single-stranded RNA analogs (or both) to repress the specific miRNAs responsible for a given disease or cancer. These analogs, including ASOs (antisense oligonucleotides), AMOs (anti-miRNA ASOs called "antagomirs"), locked nucleic acids, and antisense-technology-based small interfering RNAs, are widely and effectively used in regulation of miRNA expression [92,94–99]. But direct information is not available on the miRNAs that regulate let-7 expression; this aspect limits the scope for such a strategy. Instead, technologies are required that can effectively upregulate let-7 expression. Hence, either vector-mediated overexpression of let-7 or transient transfection of double-stranded let-7 will be the choice.

Introduction of double-stranded let-7 duplex may produce mature let-7, equivalent to the endogenous version, during Dicer processing, potentially rescuing a downregulated let-7 level. This strategy has already been successfully used [83]. Vectors containing pre–let-7–like synthetic short hairpin RNAs, driven by highly inducible Pol III promoters such as H1 and U6 [100,101] may provide high expression of let-7 from predefined transcription start and termination sites [102]. But instead of designing artificial hairpins, direct cloning of the entire natural pri–let-7 hairpin with flanking sequences into the expression vector may be a better approach—assuming that natural pre–let-7 will be a better substrate for generating mature let-7 during Dicer processing [103–107]. A pri-MIR–Pol II transgene system has been successfully used to overexpress MIR155 [104], MIR30 [108], and MIR122 [109]. This system was also found useful in expressing multiple miRNAs from a single transcript [104] and can therefore be adopted for let-7 expression too.

High-density lipoprotein conjugated siRNA has been reported to increase delivery efficacy in certain specific organs such as liver, gut, kidney, and steroid secreting organs [110]. A similar approach may therefore have the possibility to be effective in let-7 delivery as well. But the synthesis and purification of therapeutic-grade let-7 is difficult. A nanoparticle-based delivery system may prove beneficial.

Other delivery methods that have been found promising in both *in vitro* and *in vivo* conditions include lentivirus-mediated pre–let-7 oligonucleotides [36], adenovirus-mediated delivery of hairpin sequences of mature let-7 [90], cationic liposome–mediated delivery of pre–let-7 [40], and electroporation of synthetic let-7 [90]. Although such methods are at the bench level, they might be translated into therapeutic approaches in the near future.

2.7 Current Industry Status of Let-7 Therapy

Because of its potential as a cancer therapeutic, let-7 has been filed for patent protection (Australia: 2007/333109 A1; United States: 20090163430). While diagnostic companies are developing let-7–based tests for various diseases, including several cancers, pharma giants are working toward development of effective delivery systems. But let-7 restoration methods are not yet satisfactory. Asuragen (www.asuragen.com), the RNA-based therapeutic and diagnostics major with a core focus on miRNA through its subsidiary Mirna Therapeutics (www.mirnatherapeutics.com), is developing miRNA-based diagnostics and therapeutics for non-small-cell lung cancer, metastatic prostate cancer, and acute myeloid leukemia—all currently in preclinical trials. For lung cancer and acute myeloid leukemia, their main focus is let-7. Similarly, Regulus Therapeutics LLC (www.regulusrx.com) is using more than 60 miRNAs, including let-7, to develop miRNA therapeutics to treat several diseases (including cancers). Their main focus is on delivery systems and enhancement of treatment efficacy.

3. SUMMARY

Let-7 exerts its tumour suppressor and antiproliferative activities by repressing several oncogenes and by regulating key regulators of the cell cycle, cell differentiation, and apoptotic pathways. Downregulation of let-7 is a common phenomenon in several cancers, and restoration of normal let-7 expression has been found to prevent cancer growth. As a result, let-7 is a molecular marker in certain cancers and a potential therapeutic in cancer therapy. However, efficient delivery strategies have to be developed if this molecule is to be used as a therapeutic *in vivo*. Use of viral vectors, artificial virus-like particles, and nano materials may be a promising way to realize this goal, but optimization is needed. Also, a better understanding of let-7 biology and its regulatory networks is required to exploit the curative benefits of let-7 and to reduce off-target side effects.

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CLINICAL TRIAL

Aromatase expression and outcomes in the P024 neoadjuvant endocrine therapy trial

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Abstract *Background* Expression of aromatase by malignant breast epithelial cells and/or the surrounding stroma implies local estrogen production that could influence the outcome of endocrine therapy for breast cancer. *Methods* A validated immunohistochemical assay for aromatase was applied to samples from the P024 neoadjuvant endocrine therapy trial that compared tamoxifen and letrozole. The presence of aromatase expression by tumor or stromal cells was correlated with tumor response, treatment induced changes in proliferation index (Ki67), relapse-free survival (RFS) and breast cancer-specific survival (BCSS). *Results* Tumor and stromal aromatase expression were highly correlated ($P = 0.0001$). Tumor cell aromatase, as a semi-continuous score, also correlated with smaller tumor size at presentation ($P = 0.01$) higher baseline ER Allred score ($P = 0.006$) and lower Ki67 levels ($P = 0.003$). There was no significant relationship with clinical response or treatment-induced changes in Ki67. However, in a Cox multivariable model that incorporated a post-treatment tumor profile (pathological T stage, N stage, Ki67 and ER status of the surgical specimen), the presence of tumor aromatase expression at baseline sample remained a favorable independent prognostic biomarker for both RFS ($P = 0.01$, HR 2.3, 95% CI 1.2–4.6 for absent expression) and BCSS ($P = 0.008$, HR 3.76, 95% CI 1.4–10.0). *Conclusions* Autocrine estrogen synthesis may be most characteristic of smaller, more indolent and ER-rich breast cancers with lower baseline growth rates. However, response to endocrine treatment may not depend on whether the estrogenic stimulus has a local versus systemic source.

Keywords Aromatase · Letrozole · Tamoxifen · Neoadjuvant endocrine therapy

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Introduction

After the menopause, estrogen continues to be synthesized through peripheral conversion of androgenic precursors to estrone and estradiol by the CYP P450 enzyme aromatase (CYP19). Since this enzyme is widely expressed, sources