FOCUS REVIEW

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The role of let-7 in cell differentiation and cancer

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Abstract

MicroRNAs (miRNAs or miRs) are small noncoding RNAs capable of regulating gene expression at the translational level. Current evidence suggests that a significant portion of the human genome is regulated by microRNAs, and many reports have demonstrated that microRNA expression is deregulated in human cancer. The *let-7* family of microRNAs, first discovered in *Caenorhabditis elegans*, is functionally conserved from worms to humans. The human *let-7* family contains 13 members located on nine different chromosomes, and many human cancers have deregulated *let-7* expression. A growing body of evidence suggests that restoration of *let-7* expression may be a useful therapeutic option in cancers, where its expression has been lost. In this review, we discuss the role of *let-7* in normal development and differentiation, and provide an overview of the relationship between deregulated *let-7* expression and tumorigenesis. The regulation of *let-7* expression, cancer-relevant let-7 targets, and the relationship between *let-7* and drug sensitivity are highlighted.

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Introduction

The discovery of microRNAs (miRNAs or miRs) in the early 1990s has opened a new era understanding posttranscriptional regulation of genes by small RNAs (Lee *et al*. 1993). miRs are small noncoding RNAs known to repress target gene expression by binding to complementary sequences found in the 3′-untranslated region (UTRs) of target mRNAs. They form an important class of regulators that participate in diverse biological functions including development, cell proliferation, differentiation, and apoptosis (Jovanovic & Hengartner 2006, Bussing *et al*. 2008, Schickel *et al*. 2008, Stefani & Slack 2008). In respect to their diverse functions, miRs are also known to be involved in many diseases including cancer (Alvarez-Garcia & Miska 2005, Kent & Mendell 2006).

Most miRs are initially transcribed by RNA polymerase II from the genome as long primary transcripts (pri-miRs), which range in size from hundreds of bases to several kilobases (Cullen 2004). These transcripts are first processed by the microprocessor complex containing the RNase-III enzyme Drosha and the double-stranded RNA-binding protein Pasha (DGCR8) to precursor miRs (pre-miR), which are about 60–70 nucleotides long (Lee *et al*. 2003). The pre-miRs are exported from the nucleus to the cytoplasm by a Ran-GTP transporter known as exportin-5 (Yi *et al*. 2003). In the cytoplasm, the pre-miRs are further processed by a second RNase-III enzyme known as Dicer (which complexes with TRBP and Ago2) to mature miRs with a length of 20–22 nucleotides (Hutvagner *et al*. 2001, Ketting *et al*. 2001). Mature miRs are then incorporated into the miR RNA-induced silencing complex that binds to target mRNAs leading to translational repression or mRNA cleavage (Lai 2002).

The discovery of let-7 and its role in development

Although *let-7* was found as the second miR after lin-4 in *Caenorhabditis elegans* (Reinhart *et al*. 2000), its high conservation across the animal phylogeny from

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C. elegans to human provided the clue for the generality of miRs as essential regulators of gene expression in various organisms (Pasquinelli *et al.* 2000). This has led to the isolation of numerous miRs by the Ambros, Tuschl, and Bartel groups (Lagos-Quintana *et al.* 2001, Lau *et al.* 2001, Lee & Ambros 2001). Owing to recent cloning and computational efforts, the numbers of known miRs has been rapidly increasing, and to date, there are a total of 9169 mature miRs found across 103 species, of which 885 miRs are found in humans (miRBase Release 13.0 http://microrna.sanger.ac.uk/sequences). Many of these miRs are highly conserved across species, suggesting that miRs are an integral part of essential cellular processes. A good example is the *let-7* family of miRs, which is highly conserved across diverse animal species from worms to humans (Pasquinelli *et al.* 2000). Consistent with its role in regulating cell proliferation and differentiation during development in different species, the deregulation of this miR has been shown as a feature of many types of cancer, reflecting the major conserved roles of *let-7*.

Let-7 was initially identified as a heterochronic gene by forward genetics in *C. elegans* (Reinhart *et al.* 2000). During *C. elegans* development, the hypodermal skin cells known as seam cells undergo asymmetrical division at each larval stage. At the larval 4 to adult transition, these cells stop dividing, terminally differentiate, and secrete ridges called alae. *Let-7* mutant worms display abnormalities in the pattern of temporal development by reiterating their fourth larval cell fates at the adult stage (Reinhart *et al.* 2000). The seam cells fail to differentiate and exit cell cycle leading to extra seam cell divisions and lack of alae formation. Eventually, many of these mutant worms die by bursting of the vulva, thus giving the *let-7* gene its name – lethal-7. The timing of the phenotype of the *let-7* mutant corresponds with the expression of *let-7*, which can be detected at L3 and reaches maximum at L4 stage (Reinhart *et al.* 2000, Esquela-Kerscher *et al.* 2005). Studies manipulating *let-7* expression have confirmed the essential role of *let-7* in the transition from larval 4 stage to adult stage. Fusing the *let-7* gene to the *lin-4* gene and directing let-7 expression at larval stage 2 was shown to lead to precocious adult development at L4 stage, demonstrating that expression of let-7 is sufficient to specify adult fate in the worm (Hayes & Ruvkun 2006).

Other miRs that share the similar seed sequence as *let-7* in *C. elegans* are *mir-48, mir-84, mir-241, mir-265, mir-793, mir-794, mir-795*, and *mir-1821* (Roush & Slack 2008). Nothing is known about the developmental expression and functions for *mir-793, mir-794, mir-795*, and *mir-1821*. Similar to *let-7*, *mir-48*, *mir-84*, and *mir-241* are members of the heterochronic pathway, functioning in the regulation of temporal patterning at the transition from L2 to L3 stage (Abbott *et al.* 2005). This is consistent with their earlier expression, which begins at early L1 for mir-84, and at L2 for both mir-48 and mir-241, which in all cases increase over the course of development (Esquela-Kerscher *et al.* 2005, Li *et al.* 2005). Single mutants for *mir-84* and *mir-241* display normal phenotypes, whereas the *mir-48* single mutants exhibit a weak retarded defect of extra molting at the adult stage (Abbott *et al.* 2005). The penetrance of this retarded molting defect was enhanced in *mir-48*/*mir-84* double mutants. The *mir-48*/*mir-241* double mutants have a retarded seam cell phenotype in which seam cells undergo an extra cell division during the L3 stage. These double mutants also display incomplete alae formation and lethality associated with vulval bursting at the L4 to adult transition. These defects occur with higher penetrance in the triple mutants compared to the double mutants, indicating functional cooperation among these three miRs (Abbott *et al.* 2005).

Let-7, mir-48, mir-84, and mir-241 are expressed at different times during development (Reinhart *et al.* 2000, Esquela-Kerscher *et al.* 2005). Lin-4 and nuclear receptor dauer formation-12 (DAF-12) mutants have reduced levels of the mature forms of these miRs (Esquela-Kerscher *et al.* 2005). Recently, DAF-12 and its steroid ligand have been shown to directly activate promoters of *mir-84* and *mir-241*, which leads to downregulation of their target, hunchback-like (HBL)-1, allowing L2 to L3 transition (Bethke *et al.* 2009). DAF-12 itself is a target of let-7 at later stages, indicating that feed forward and feedback loops are involved in driving different stage transitions (Grosshans *et al.* 2005). While DAF-12 seems to be a nematode-specific let-7 target, other targets such as RAS (homolog of *let-60*) and TRIM71 (*lin-41*) are conserved among various organisms (Kloosterman *et al.* 2004, Johnson *et al.* 2005, Schulman *et al.* 2005, Lin *et al.* 2007, Yu *et al.* 2007*a*, O'Farrell *et al.* 2008).

Recent studies in *Drosophila* have shown that *let-7* also functions as a heterochronic gene in this species (Caygill & Johnston 2008, Sokol *et al.* 2008). In *Drosophila*, there is only a single *let-7* gene (Lagos-Quintana *et al.* 2001), and it becomes expressed at the end of the third larval instar stage and peaks in pupae during metamorphogenesis (Pasquinelli *et al.* 2000, Bashirullah *et al.* 2003). Like *C. elegans*, *Drosophila* undergo a series of molting processes in their development, and a pulse

of ecdysone is released before each molting stage. The temporal expression of *let-7* coincides with the release of ecdysone, but whether ecdysone regulates the expression of *let-7* is still unclear (Sempere *et al*. 2002, Bashirullah *et al*. 2003). Recent studies have shown that *let-7* mutants display a temporal delay in the terminal cell cycle exit in the wing and also have defects in maturation of neuromuscular junctions at adult abdominal muscles (Caygill & Johnston 2008, Sokol *et al*. 2008). The mutants exhibit clear juvenile features in their neuromusculature, and these lead to defects in adult behaviors such as flight, motility, and fertility (Sokol *et al*. 2008). Therefore, let-7 expression ensures the appropriate remodeling of the abdominal neuromusculature during the larval to adult transition serving as a conserved regulator of events necessary for the transition from juvenile to adult life stages.

There are 14 and 13 different *let-7* family members in mouse and human respectively (Roush & Slack 2008). In human, these different members are *let-7a-1, 7a-2, 7a-3, 7b, 7c, 7d, 7e, f7-1, 7f-2, 7g, 7i, mir-98, and mir-202* (Ruby *et al*. 2006). Among the members, *let-7a* has identical sequence across various animal species from *C. elegans* to human. The increase in let-7 expression in late developmental stages has been reported in many organisms (Sempere *et al*. 2002, Lancman *et al*. 2005, Liu *et al*. 2007, Wulczyn *et al*. 2007). Previously, studies have shown the expression patterns of let-7 in vertebrates during development; however, the direct contribution of *let-7* in development has not been demonstrated (Lancman *et al*. 2005, Schulman *et al*. 2005, Wulczyn *et al*. 2007). This is probably because vertebrate *let-7* family members are likely to have redundant roles, and it is technically challenging to knock out all the members of the *let-7* family in the same animal.

As demonstrated by its role in seam cell differentiation at the larval to adult transition in *C. elegans*, a major role of *let-7* is to promote differentiation of cells. In mammals, let-7 levels increase during embryogenesis and during brain development (Schulman *et al*. 2005, Wulczyn *et al*. 2007). Let-7 is undetectable in human and mouse embryonic stem cells, and the level of let-7 increases upon differentiation (Thomson *et al*. 2004, 2006, Wulczyn *et al*. 2007). This high expression of let-7 is then maintained in various adult tissues (Sempere *et al*. 2004, Thomson *et al*. 2004). Conversely, the reduction of let-7 levels has been found in many human cancers, which is reflective of the reverse embryogenesis process that occurs during tumorigenesis (Park *et al*. 2007). As we will discuss in the following, while let-7 was initially viewed as one single activity, emerging data suggest that the let-7 family contains miRs with different activities. We will therefore wherever possible name the specific let-7 family members throughout this review.

Deregulation of let-7 family members in different cancer types

Let-7 is widely viewed as a tumor suppressor miR. Consistent with this activity, the expression of let-7 family members is downregulated in many cancer types when compared to normal tissue and during tumor progression. For some forms of cancer, most or all let-7 family members appear to be downregulated (Takamizawa *et al*. 2004, Dahiya *et al*. 2008, O'Hara *et al*. 2009). However, downregulation of specific family members in various cancers has also been described and is summarized in Table 1. The loss of let-7 family members also has prognostic value as it indicates poor survival. A general downregulation of let-7 was found to correlate with poor survival in lung cancer (Takamizawa *et al*. 2004). Specifically, this was also reported for let-7a-2 (Yanaihara *et al*. 2006). Low expression of let-7d (combined with low miR-205 expression) was found in head and neck squamous cell carcinoma (HNSCC) patients, and was predictive of poor survival (Childs *et al*. 2009), and a combined loss of let-7d with an increase in expression of the let-7 target high mobility group 2A (HMGA2) was indicative of poor survival in ovarian cancer (Shell *et al*. 2007).

Although less frequent, upregulation of certain let-7 family members has also been observed, suggesting that let-7 does not play a tumor suppressor function under all circumstances and/or in all tissues (Table 1). The upregulation of let-7b and let-7i was associated with high grade transformation in lymphoma (Lawrie *et al*. 2008), indicating that increased expression of let-7 family members could be used as a prognostic marker to identify patients at risk of high grade transformation, or for higher grade cancer. The most detailed mechanistic analysis of an up-regulated let-7 family member was performed for let-7a-3. Hypomethylation of the let-7a-3 locus was found to cause higher expression of let-7a-3 in epithelial ovarian cancer (Lu *et al*. 2007) and lung cancer (Brueckner *et al*. 2007). Hypomethylation did not only cause increased expression of let-7a-3, but subsequently deregulated the expression of other genes, including oncogenes and genes involved in cell proliferation, adhesion, and differentiation (Brueckner *et al*. 2007).

The conflicting data on the deregulation of *let-7* in various cancers indicate that the function of the *let-7*

Table 1 Changes in the expression of *let-7* family members in human cancer

Family member	Cancer type downregulated	Cancer type up-regulated	Reference
7a	Breast		Sempere *et al.* (2007)
	Lung		Takamizawa *et al.* (2004), Johnson *et al.* (2005), and Yanaihara *et al.* (2006)
	Melanoma		Muller & Bosserhoff (2008)
	Pancreatic		Torrisani *et al.* (2009)
	PPNAD		Iliopoulos *et al.* (2009)
		Lung[a]	Brueckner *et al.* (2007)
		Lymphoma	Nie *et al.* (2008)
		Ovarian[a]	Lu *et al.* (2007)
7b	ALL		Mi *et al.* (2007)
	Melanoma		Schultz *et al.* (2008)
	Ovarian		Nam *et al.* (2008)
	PPNAD		Iliopoulos *et al.* (2009)
	Prostate		Ozen *et al.* (2008)
	Retinoblastoma		Huang *et al.* (2007)
		GIST	Subramanian *et al.* (2008)
		Lymphoma	Lawrie *et al.* (2008)
7c	Burkitt lymphoma[b]		Leucci *et al.* (2008)
	Lung		Johnson *et al.* (2005), and Tokumaru *et al.* (2008)[c]
	PPNAD		Iliopoulos *et al.* (2009)
	Prostate		Ozen *et al.* (2008)
7d	HNSCC		Childs *et al.* (2009)
	Ovarian		Shell *et al.* (2007), and Dahiya *et al.* (2008)
	Prostate		Ozen *et al.* (2008)
7e	Ovarian		Dahiya *et al.* (2008)
	Prostate		Ozen *et al.* (2008)
7f	Lung		Takamizawa *et al.* (2004)
	Ovarian		Dahiya *et al.* (2008)
	Prostate		Ozen *et al.* (2008)
	Sarcoma		Subramanian *et al.* (2008)
		Breast	Yan *et al.* (2008)
7g	Lung		Johnson *et al.* (2005)
	PPNAD		Iliopoulos *et al.* (2009)
	Prostate		Ozen *et al.* (2008)
7i	Ovarian		Yang *et al.* (2008*b*)
	Prostate		Ozen *et al.* (2008)
		HNSCC	Chang *et al.* (2008*a*)
		Lymphoma	Lawrie *et al.* (2008)
7b*		Mesothelioma	Guled *et al.* (2009)
7e*	Mesothelioma		Guled *et al.* (2009)
miR-98		Breast	Yan *et al.* (2008)

PPNAD, primary pigmented nodular adrenocortical disease; ALL, acute lymphoblastic leukemia; GIST, gastrointestinal stromal tumors; HNSCC, head and neck squamous cell carcinoma.
[a]*Let-7a-3*.
[b]Negative for c-myc translocation.
[c]Caused by genetic loss of 21q11–q21.

family is not clearly defined, or that individual *let-7* family members can have different activities. While the different family members are currently being viewed as targeting a highly overlapping set of targets, it is conceivable that the 13 *let-7* genes do not exist simply to have different functions in different tissues but because two *let-7* family members could have different functions in the same cell. Consistent with this prediction are occasional reports of cancers in which certain let-7 species are up-regulated, while others are lost. An example of this is a report on malignant mesothelioma, where let-7b* was found to be highly expressed but let-7e* was severely reduced (Guled *et al*. 2009). Cases such as this suggest that let-7

family members are potentially subject to differential regulation within the same cell, and an important area the miR field will need to address is whether different family members indeed have specific activities in a particular cell type, or whether tissue-specific regulation is the most important mechanism utilized to obtain specific let-7-mediated cellular outcomes regardless of which let-7 member is involved.

Cancer-relevant let-7 targets

The first mammalian target of let-7 was identified by virtue of evolutionary sequence conservation between the nematode *C. elegans* and humans. Using a computational screen for *C. elegans* 3′-UTR sequences containing let-7 family complementary sites (LCS), Johnson *et al.* (2005) identified *let-60* as a top-scoring candidate whose human ortholog is the three RAS oncogenes that are frequently deregulated in many human cancers. It was determined that let-60 expression is tightly and directly regulated by miR-84 in *C. elegans* vulval cells, and it was also demonstrated that human let-7 does specifically target RAS in human cancer cells. The same group determined that *let-7* family members 7a, 7c, and 7g are significantly decreased in lung cancer tumors, and that many *let-7* family members are located in genomic regions frequently deleted in lung cancer patients. This evidence, together with the fact that RAS is frequently up-regulated in human lung tumor samples, suggested a pivotal role for the let-7 family in the suppression of oncogenic RAS proteins *in vivo*. This direct targeting of RAS by let-7 was confirmed in nonsmall cell lung cancer (NSCLC), where it was demonstrated in a mouse model that let-7g inhibited tumor growth via suppression of RAS (Kumar *et al.* 2008).

Single nucleotide polymorphisms (SNPs) within the 3′-UTRs of target genes have only recently been implicated in miR-mediated control of gene expression. Weidhaas *et al.* investigated whether Kirsten rat sarcoma viral oncogene homolog (KRAS) LCS contained SNPs that could affect miR binding (Chin *et al.* 2008). They identified a variant allele in one of the ten KRAS LCS sequences (KRAS LCS6), which was found to be significantly higher in NSCLC patients than in healthy individuals (5.8% healthy, 20.3% NSCLC as per Yale University DNA collection) and predicted for an increased risk of developing NSCLC for moderate smokers. Another report concerning the LCS6 SNP in RAS came from studies of HNSCC, where this allele is associated with poorer patient survival but does not confer increased risk to HNSCC. Survival was reduced most significantly in oral cancer patients, suggesting the LCS6 polymorphism might be important when considering therapeutic approaches for these patients (Christensen *et al.* 2009). Recently, the Kras 3′-UTR SNP was also linked to an increased risk of developing ovarian cancer (J Weidhaas, I Babar, S M Nallur, P Trang, S Roush, M Boehm, E Gillespie, F J Slack, personal communication).

The second major let-7 target identified was HMGA2, which is a chromatin-associated nonhistone protein capable of modulating chromatin architecture and thus affecting transcription. It is a gene with an oncofetal pattern of expression and is widely expressed in undifferentiated embryonal tissues but undetectable in normal adult tissues. Its importance during animal development was revealed by *hmga2* knockout mice, which display mesenchymal tissue hypoplasia leading to a pygmy phenotype (Zhou *et al.* 1995). In addition to its role during embryogenesis, HMGA2 is expressed in both benign and malignant tumors mostly due to deregulation via chromosomal aberrations on 12q13–15. These rearrangements are known to truncate the HMGA2 open reading frame (ORF) releasing the DNA-binding domain from the adjacent acidic domain, and this rearrangement is associated with oncogenic transformation. However, in addition to ORF truncation, the separation of the regulatory 3′-UTR region from the intact HMGA2 coding sequence is also known to have similar neoplastic effects in human tumors and a transgenic mouse model, suggesting that control of HMGA2 expression via the 3′-UTR is an important control mechanism. HMGA2 translocations also recurrently involve fusion of the HMGA2 3′-UTR to known tumor suppressor genes such as FHIT, RAD51L1, and HEI10, and let-7-mediated repression of these genes might enhance the oncogenic potential of such rearrangements (Mayr *et al.* 2007).

Multiple lines of initial evidence suggested that HMGA2 might be a direct let-7 target gene. The 3′-UTR of HMGA2 contains multiple let-7 complementarity sites; it is downregulated during embryogenesis coinciding with concomitant let-7 upregulation, and it was shown to be up-regulated in lung cancer – a cancer known for its lowered let-7 expression. Data published by three groups (Lee & Dutta 2007, Mayr *et al.* 2007, Shell *et al.* 2007) confirmed HMGA2 as a direct let-7 target both *in vitro* and *in vivo*. Mayr *et al.* demonstrated that the truncated form of HMGA2 is uncoupled from let-7-induced growth suppression, and that this protein has oncogenic properties when expressed in NIH3T3 cells, which express endogenous levels of let-7. The same aggressiveness was also seen *in vivo* with injection of cells expressing the truncated HMGA2 protein. It was demonstrated in ovarian

cancer patients that the ratio of let-7 to HMGA2 was useful as a prognostic marker, with a lower ratio predicting shorter overall survival (Shell *et al*. 2007). Interestingly, there are also reports linking RAS with HMGA2, in the maintenance of a mesenchymal phenotype. In a study by Watanabe *et al*. (2009), oncogenic RAS signaling was found to induce HMGA2 expression and thus inhibit the epithelial phenotype of pancreatic cancer cells.

In addition to aberrant expression in lung and ovarian cancer, HMGA2 is frequently elevated in uterine leiomyomas where higher expression of HMGA2 and lower expression of let-7 correlate with larger tumor size (Peng *et al*. 2008). HMGA2 overexpression is also seen in pituitary adenomas, which are neoplastic intracranial tumors that frequently have deregulated let-7 expression. In a recent study, 39% of pituitary adenomas analyzed had significantly higher HMGA2 expression, which correlated with higher tumor grade and tumor invasiveness (Qian *et al*. 2009). A significant inverse correlation between HMGA2 and let-7 expression was observed in these tumors, suggesting that loss of let-7 expression contributes to the aggressive phenotype of these cancers via re-expression of HMGA2.

As mentioned above, *hmga2* knockout mice were found to display many of the principal features of the pygmy phenotype (Zhou *et al*. 1995). The stunted growth of these animals was also found to be semi-dominant with heterozygous animals weighing 80% of their wild-type littermates. These data are in accordance with more recent data where HMGA2 expression levels have been shown to correlate with larger tumor size (Peng *et al*. 2008), and a truncated HMGA2 has been shown to correlate with larger body size (Nishino *et al*. 2008) and reduced adult human height (Lettre *et al*. 2008). While this phenotype can be explained in multiple ways, it is potentially suggestive of a defect in the stem cell compartment. In an interesting new development, evidence has begun to link lack of let-7 expression to the maintenance of the stem cell phenotype (Peter 2009). A study investigating breast cancer tumor-initiating cells (T-ICs) determined that they are let-7 low, and that HMGA2 is important for maintaining their pluripotency (Yu *et al*. 2007*a*). It was also determined that low let-7 expression characterizes mouse mammary epithelial progenitor cells that have many of the characteristics of murine stem cells and can completely repopulate the mammary tree from single cells (Ibarra *et al*. 2007). This evidence suggests that major let-7 targets, including HMGA2, might be participating in maintenance of a stem cell phenotype. Interestingly, HMGA2 was the major differentially expressed gene found using a genome-wide analysis of murine neural stem cells during aging (Nishino *et al*. 2008). The expression of HMGA2 was reduced in neural stem cells in an age-dependent manner, and this coincided with induction of let-7b. HMGA2 expression was not necessary for stem cell formation, but it was paramount to maintaining self-renewal potential of young stem cells. This maintenance of stemness was dependent on HMGA2 negatively regulating $p16^{Ink}$ and $p19^{Arf}$, two important negative regulators of cell cycle progression.

The regulation of HMGA2 by let-7 is not only pivotal in maintaining tissue homeostasis, but it has recently been implicated in other cellular functions. Mouse adipogenesis relies on the clonal expansion of undifferentiated cells upon adipogenic stimuli, cell cycle exit, and subsequent terminal differentiation. Recently, it was demonstrated that during transition from expansion to differentiation, let-7c and let-7b are significantly up-regulated and maintained at elevated levels in mature adipocytes (Sun *et al*. 2009). This differentiation relies on loss of HMGA2, which had previously been implicated in adipogenesis. Mice lacking HMGA2 have reduced adipose tissue, whereas animals overexpressing a truncated HMGA2 have an increased amount of fat tissue (Zhou *et al*. 1995). Indeed, in adipocytes treated with let-7a, the most markedly reduced gene is HMGA2, and siRNA-mediated knockdown of HMGA2 prevents adipocyte differentiation. Determination of adult height in humans is a highly variant polygenic trait regulated by multiple genes by a largely unknown synergistic mechanism. Genome wide association studies were used to identify ten loci associated with height determination where two SNPs were robustly associated with adult height variation (Lettre *et al*. 2008). One of these SNPs is located in the 3′UTR of HMGA2 very close to a binding site of let-7 possibly abolishing let-7 binding to this site. In addition to the highly predictive SNP in HMGA2, three other let-7 targets are among the ten loci identified. These additional genes are CKD6 (important in cell cycle regulation), DOT1L (histone methyltransferase), and LIN28B (let-7 regulation).

Our group has recently identified 12 cancer-relevant let-7 regulated oncofetal genes (LOGs; Boyerinas *et al*. 2008). This study looked at the overlap between genes predicted to be let-7 targets by target prediction algorithms, genes experimentally shown to be down-regulated when let-7 was overexpressed in two cancer cell lines, and genes downregulated at the time during murine embryonic development when let-7 is drastically up-regulated. Twelve genes met these criteria, and eight of the twelve had been previously implicated in cancer formation or development. LOG #1 was HMGA2, LOG #2 was IGF2 mRNA-binding protein

(IMP)-1, and LOG #3 was LIN28B (which will be discussed in detail later). IMP-1 (also known as insulin-like growth factor protein 1 and CRD-BP) is an RNA-binding protein with a known oncofetal pattern of expression that had previously been linked to multiple forms of cancer. We experimentally validated IMP-1 as a let-7 target and determined that it was an important target gene for let-7-mediated inhibition of growth and invasiveness in let-7 low cancer cell lines. We hypothesize that many of the LOGs become re-expressed in cancers that lose let-7 expression, and that this process can be considered a form of reverse embryogenesis.

Though the vast majority of let-7 targets seem to have oncogenic properties with let-7 acting as a tumor suppressor, recent reports also attribute an oncogenic role to let-7a. Caspase-3, a pivotal protease activated during apoptosis, contains let-7a seed matches, and it was determined that let-7a specifically targets caspase-3 mRNA in cancer cell lines (Tsang & Kwok 2008). Taken together with previously published data where the let-7a-3 locus was found to be hypomethylated in human lung adenocarcinomas (Brueckner *et al*. 2007), these results raise the possibility that let-7a acts in an oncogenic manner.

Another scenario where let-7 acts in an oncogenic fashion involves the transcriptional repressor Blimp-1 (also known as PRDM1). This factor is important for terminal differentiation of lymphocytes and epidermal cells, and is considered a crucial determinant in plasma cell differentiation. Loss of Blimp-1 expression has been described as a characteristic event in Hodgkin lymphoma and other human lymphomas. The reduced expression of Blimp-1 was determined to be the result of upregulation of two miRs able to engage the Blimp-1 mRNA; the endogenous miRs miR-9 and let-7a (Nie *et al*. 2008). Downregulation of Blimp-1 by let-7a suggests an oncogenic role for this miR in these cells, as this enables inappropriate and potentially neoplastic cell division. There are, however, reports on let-7a conferring anti-neoplastic protection by counteracting c-Myc-induced growth in Burkitt lymphoma. Exogenously overexpressed let-7a caused a significant repression of Myc levels, and this led to reduced proliferation in lymphoma cells *in vitro* (Sampson *et al*. 2007). These data suggest that the cellular response to let-7a may be dependent on cell context in much the same way that p53 activation elicits drastically different results in different cell types.

There are some interesting recent reports detailing negative feedback loops involving Dicer, the RNAse III nuclease known to process pre-miR, and let-7. Identified as a putative let-7d target by the target algorithm PicTar, the inverse correlation of let-7a and Dicer was confirmed in a panel of 20 cell lines whilst Drosha, the RNAse involved in the processing of pri-miR molecules, did not correlate with let-7 expression (Tokumaru *et al*. 2008). Dicer was confirmed as a direct target of let-7a, and exogenous expression of let-7a, -7c, or -7d was shown to be sufficient for the marked downregulation of Dicer mRNA and protein. This decrease in Dicer does not only negatively affect the processing of let-7 family members but rather has a broad effect on the processing of many other miRs in both normal and cancer cell lines. Importantly, reduced expression of Dicer has been associated with poor prognostic outcome in NSCLCs (Tokumaru *et al*. 2008). The regulation of Dicer through its 3′-UTR is not the only mechanism by which the let-7 family controls the expression of this nuclease. In a screen for putative miR-targeting sites in the ORFs of protein-coding genes, LCS's were found to be the single most highly conserved sequences identified. Among genes containing these ORF targeting motifs, the Dicer ORF was found to contain three let-7 target sites leading to it being efficiently downregulated by exogenous expression of let-7 (Forman *et al*. 2008).

Repression of let-7 targets does not rely solely on the activity of the miR alone. The transcription factor c-Myc is also repressed in an interdependent manner by let-7b/c and the RNA-binding protein Human antigen R (HuR; Kim *et al*. 2009). This regulation relies on the binding of HuR to the 3′-UTR of c-Myc leading to the recruitment of let-7 loaded RISC complexes to the adjacent let-7 target site. This concerted binding leads to the efficient degradation of the c-Myc mRNA in an Ago2-dependent way. This additional layer of regulation to let-7 targeting is likely only one example of protein-mediated control of miR function.

Let-7 has been linked to a number of cellular responses that could be exploited for cancer therapy, including regulation of cellular proliferation (Gu & Iyer 2006). Overexpression of let-7a, 7b, and 7c were shown to inhibit the growth of cancer cell lines (Johnson *et al*. 2007), and one way that let-7 negatively regulates growth is by inhibiting protein translation (Ding *et al*. 2008). A mechanism to explain this activity is the specific targeting of eIF4F by let-7a (Mathonnet *et al*. 2007). However, an alternative mechanism involves degradation of various cell cycle regulators (Johnson *et al*. 2007), which was specifically demonstrated for let-7b in melanoma cells (Schultz *et al*. 2008). However, this activity of let-7 may not be tumor cell specific since in primary fibroblasts let-7b was shown to cause G2/M arrest through targeting

Cdc34 resulting in the stabilization of the Wee1 kinase (Legesse-Miller *et al*. 2009). Finally, let-7f and 7g have been shown to be up-regulated in cells with induced premature senescence (Maes *et al*. 2009), and let-7f was found to be up-regulated during replicative senescence of mesenchymal stem cells (Wagner *et al*. 2008). Let-7's role in regulating senescence is consistent with the function of one of its main targets, HMGA2, in this process (Narita *et al*. 2006).

First attempts to utilize the apparent anticancer activity of let-7 for therapeutic purposes have been made by two groups who used let-7b, 7c, 7g, and let-7g respectively to slow tumor formation in a mouse lung cancer model (Esquela-Kerscher *et al*. 2008, Kumar *et al*. 2008). However, a more recent study on pancreatic cancer suggested that while let-7a affected proliferation of cancer cells *in vitro*, this did not translate into reduced tumor growth *in vivo* (Torrisani *et al*. 2009). It is important to note that the activity of let-7 to reduce tumor growth *in vivo* is dependent upon the particular cellular context. While in proliferating cells it inhibits translation, in cells arrested in the cell cycle let-7 can actually induce translation (Vasudevan *et al*. 2007). These data demonstrate that more research is needed into the specific activities of the let-7 family before let-7 can be utilized in a therapeutic context.

The regulation of let-7 expression in cancer

It has been reported that miRs are globally down-regulated during cancer formation (Lu *et al*. 2005, Thomson *et al*. 2006). In addition to this global effect, expression of miRs with specific tumor suppressor activities is lost in many forms of cancer. Let-7 is a marker of fully differentiated cells, and it is undetectable in stem cells (Ibarra *et al*. 2007). Many of the targets of let-7 have activities in stem cells, and the expression of let-7 must therefore be carefully controlled (Peter 2009). Different levels of regulation in the biogenesis of let-7 have recently emerged revealing an intricate regulatory loop comprising let-7 and some of its targets with stem cell activity.

First evidence of extensive posttranscriptional regulation of let-7 came from studies of mouse embryonic development. Both in the developing brain and the entire mouse, mature let-7 was found to be absent early during development (Thomson *et al*. 2006, Wulczyn *et al*. 2007). In contrast, the primary transcript of let-7 was expressed at high levels at these stages. An inverse situation was found in cancer where cancer cells that had lost expression of mature let-7 still expressed the primary transcript (Thomson *et al*. 2006). These data suggested the existence of a regulatory mechanism that affected the biogenesis of let-7 either at the Drosha or Dicer level, or at the level of export of pre-let-7 to the cytosol. Because this effect was selective for let-7 family members, it was assumed that a protein factor could selectively inhibit the processing of let-7 during development. Two groups set out to biochemically identify this factor from differentiating P19 embryonic stem cells by coupling pre-let-7 molecules to beads and pulling down proteins that bound to the loop region of let-7 (Newman *et al*. 2008, Viswanathan *et al*. 2008). In both cases, two proteins were identified using mass spectrometry – Lin28 and Lin28B. Both these proteins were found to be selective in their ability to block processing of let-7 family members (Fig. 1). However, the two groups came to different conclusions as to the mechanisms of action. The Hammond group tested various let-7 family members and concluded that Lin28/Lin28B act at the level of Drosha, whereas the Gregory group, mostly focusing on let-7a and g, identified Dicer as the site of action. In any case, the identification of Lin28/Lin28B as a regulator of let-7 processing represents a major breakthrough in our understanding of how let-7 expression is regulated during both embryonic development and cancer progression. In a subsequent biochemical study, it was shown in detail how Lin28 binds to the terminal loop region of let-7g (Piskounova *et al*. 2008). Both cold shock domains and both zinc finger domains in Lin28 are involved in the binding, resulting in more than 90% inhibition of let-7g processing upon upregulation of Lin28.

More recently, a third mechanism of how Lin28/Lin28B block let-7 processing was discovered (Fig. 1). It was found that in cells with blocked let-7 processing, pre-let-7 was posttranscriptionally modified and carried a 14-nt long extension mostly comprising uridine (Heo *et al*. 2008). This polyuridylation resulted in degradation of pre-let-7. Recently, the terminal uridylyl transferase (TUTase) was identified as TUT4 (also known as ZCCHC11; Hagan *et al*. 2009, Heo *et al*. 2009). We and others recently identified Lin28B as one of the main let-7 targets (Boyerinas *et al*. 2008, Rybak *et al*. 2008), and it was shown that let-7 and Lin28 are part of a double negative feedback loop regulating expression of let-7, which is conserved from worms to humans (Rybak *et al*. 2008, Peter 2009). Recently, the *C. elegans* TUTase that uridylates let-7 was identified as PUP-2 (an ortholog of TUT4; Lehrbach *et al*. 2009). The negative feedback loop that involves Lin28/Lin28B and let-7 also includes c-Myc, which is both a target of let-7 itself (Sampson *et al*. 2007) as well as a potent regulator of the

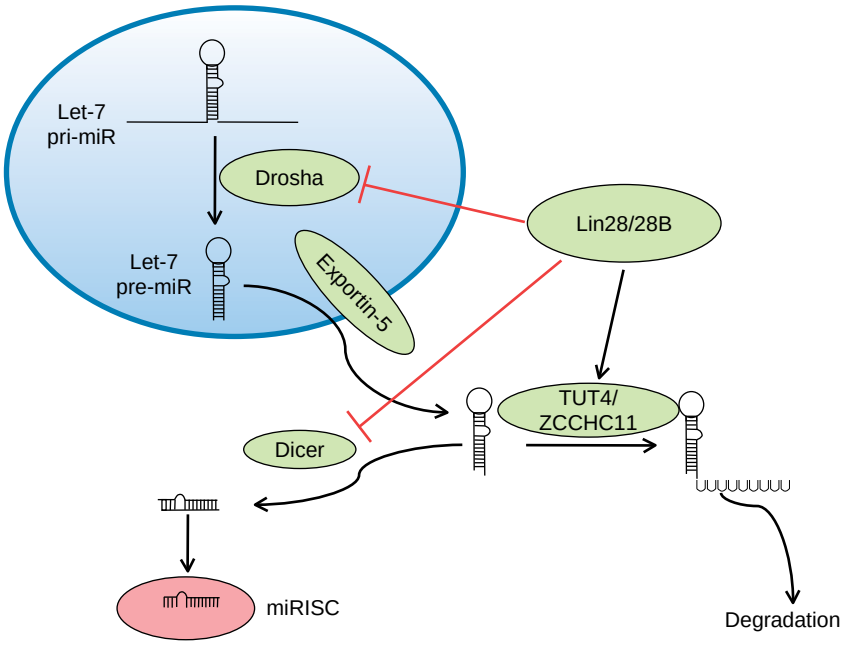

Figure 1 Regulation of the biogenesis of let-7 by Lin28/Lin28B by three different mechanisms. For details see text.

expression of various let-7 family members (Chang *et al*. 2008*b*). Recently, it was demonstrated that c-Myc also targets Lin28B causing repression of let-7 without affecting its transcriptional expression adding to the complexity of the feedback loop (Chang *et al*. 2009, Dangi-Garimella *et al*. 2009).

The importance of the let-7 regulators Lin28 and Lin28B is also emerging from studies demonstrating upregulation of Lin28 or Lin28B in various forms of cancer for which a downregulation of let-7 has been shown. The first demonstration of Lin28B being up-regulated in a human cancer was made for hepatocellular carcinoma (HCC; Guo *et al*. 2006). In ovarian cancer, an inverse relationship between the expression of let-7a and Lin28B was found, and Lin28B was identified as an unfavorable prognostic marker (Lu *et al*. 2009). Most recently, Lin28 and Lin28B were reported to be up-regulated in various forms of cancer including Wilm's tumor, HCC, chronic myeloid leukemia, and ovarian cancer with an overall incidence of about 15% (Viswanathan *et al*. 2009). Their overexpression facilitated cellular transformation in various *in vitro* systems and promoted tumor growth in mouse tumor models. In another study, it was shown that Lin28 enhances metastasis (Dangi-Garimella *et al*. 2009).

While Lin28 and Lin28B are important in the regulation of let-7 processing, they are also active as stem cell factors, and Lin28 has been shown together with three other factors (Oct4, Sox2, and Nanog) to be sufficient to reprogram somatic fibroblasts to become pluripotent stem cells (Yu *et al*. 2007*b*). Consistently, Lin28 was also reported to be involved in the development of a rare founder population of germ cells (West *et al*. 2009) and to be frequently up-regulated in germ cell tumors. In fact, Lin28 and Lin28B were more consistently up-regulated in such tumors than even the very potent stem cell factor Oct4. All these reports point at a function of Lin28/Lin28B as powerful regulators of stem cells and germ cells. There is evidence that some of these activities involve the regulation of let-7.

While Lin28 and Lin28B are emerging as major regulators of let-7 activity, transcriptional regulation of let-7 has also been reported, and it was shown that pri-let-7 is negatively regulated by a pathway controlled by the microtubulin-associated kinase DCAMKL-1 (Sureban *et al*. 2009). Furthermore, it was recently demonstrated that Lin28 has additional activities that could contribute to its activity as a stem cell factor. It was shown that Lin28 promotes production of the replication-dependent histone mRNA H2a and of cyclin A in mouse embryonic stem cells (Xu & Huang 2009). Hence, the regulation of let-7 levels is unlikely the only activity of Lin28 and Lin28B.

Posttranslational regulation of miR expression is not limited to let-7 (Lu *et al*. 2005, Thomson *et al*. 2006), and it is widely expected that Lin28 and Lin28B only represent one example of a new class of regulators of miR biogenesis. In fact, other factors are emerging that

have been demonstrated to control let-7 expression. A complex of nuclear factor 90 (NF90) and NF45 was shown to negatively regulate expression of miRs. While NF90-NF45 had a preference for let-7a over miR-21, it also affected processing of other miRs (Sakamoto *et al*. 2009). The identification of Lin28 and Lin28B is therefore only the tip of the iceberg, and a detailed knowledge of the factors regulating various miRs under different physiological and disease conditions may allow us to correct expression of dysregulated miRs in diseases such as cancer.

miRs and drug sensitivity

Chemotherapy is one of the most frequently utilized treatment modalities for various forms of human cancer. Unfortunately, the majority of patients in most forms of cancer relapse within 5 years, and recurrent disease is frequently much more resistant to treatment via chemotherapeutic agents. Understanding the mechanisms via which drug resistance evolves in treatment-refractory cancers is critically important in the fight to reduce cancer-related mortalities. Changes in miR expression profiles, due to their profound effect on gene expression signatures, are emerging as an intriguing mechanism for the development of chemoresistance in many cancers (Fig. 2).

A group of miRs has been implicated in modulation of survival pathways and/or apoptotic response in cancer cells. MiR-15b and miR-16 target the anti-apoptotic protein Bcl2 and reduce its expression level (Xia *et al*. 2008), while miR-1 overexpression sensitizes A549 lung cancer cells to doxorubicin-induced death by reducing levels of the anti-apoptotic protein Mcl-1 (Nasser *et al*. 2008). At least three miRs modulate the PTEN/Akt survival pathway; miR-214 expression induces cell survival and resistance to cisplatin by specifically targeting the PTEN 3′-UTR (Yang *et al*. 2008*a*), miR-205 specifically targets the 3′-UTR of the HER3 receptor and restores sensitivity to Gefitinib via reduction of HER3 protein levels and inhibition of Akt signaling (Iorio *et al*. 2009), and miR-21 expression activates Akt-dependant survival signaling in cholangiocarcinoma cell lines (Meng *et al*. 2006). Additionally, miRs-221 and 222 maintain a TRAIL-resistant phenotype by targeting p27 in NSCLC (Garofalo *et al*. 2008). MiR-143 expression sensitizes Jurkat leukemia cells to Fas-mediated apoptosis by directly targeting ERK5 (Akao *et al*. 2009). p53 transcriptionally induces miR-34a

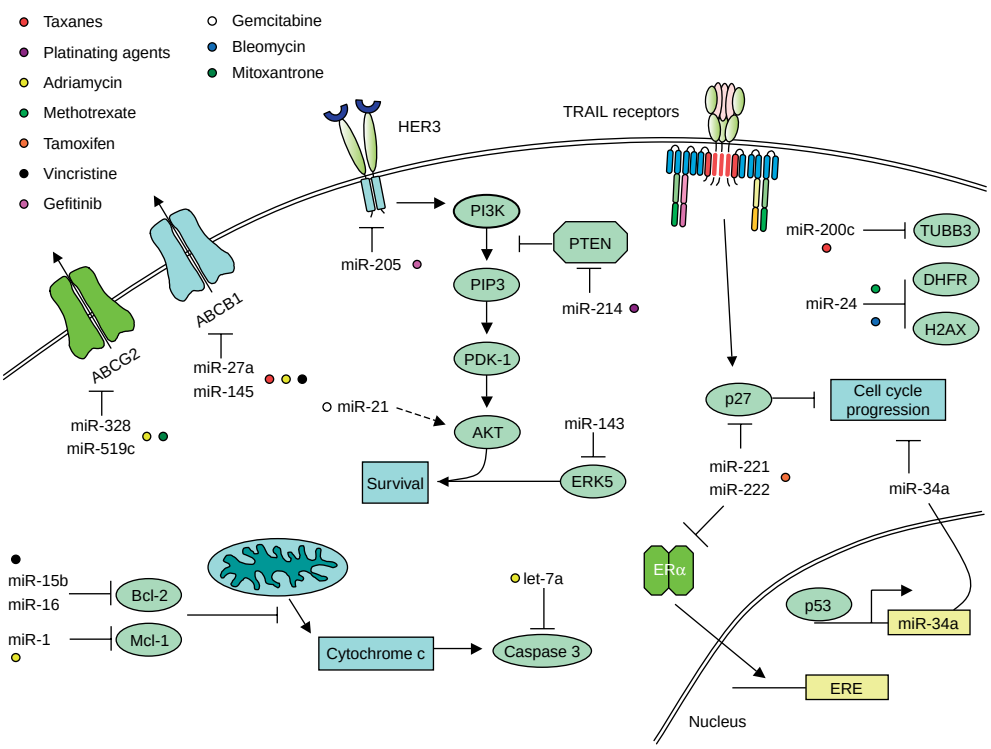

Figure 2 Scheme to illustrate how miRs affect drug sensitivity of cancer cells. In each case, the miRs are not direct inhibiting proteins but are acting by regulating the mRNAs that code for the proteins shown. The drug resistance that was reported to be affected by a specific miR is indicated by a colored dot next to the miR. For details see text.

expression, which in turn leads to cell cycle arrest and apoptosis induction (Chang *et al*. 2007).

A second group of miRs has been demonstrated to alter cellular response to a specific drug or class of drugs independently of survival or apoptotic signaling (Fig. 2). MiR-200c targets TUBB3, a tubulin isotype that is generally found only in neurons but is expressed in some cancers with resistance to microtubule-binding agents (Cochrane *et al*. 2009). MiR-24 targets the 3′-UTR of dihydrofolate reductase (DHFR), and a SNP in the DHFR 3′-UTR that abolishes this regulation leads to methotrexate resistance as a result of DHFR overexpression (Mishra *et al*. 2007). MiR-24 also sensitizes terminally differentiated blood cells to DNA damage by targeting the 3′-UTR of H2AX and inhibiting the double-strand break response (Lal *et al*. 2009). MiRs-221 and 222 specifically target the 3′-UTR of estrogen receptor-α and induce tamoxifen resistance in breast cancer cell lines (Lal *et al*. 2009). Many cancers, especially recurrent cases that occur after prior chemotherapeutic treatment, become resistant to specific classes of drugs – a situation known as multi-drug resistance. This phenotype is mediated in many cases by the overexpression of transmembrane drug efflux pumps that transport drugs from the cytosol to the extracellular space. Recent evidence suggests that expression of two of these pumps, ABCB1 (MDR1) and ABCG2 (BCRP), is directly regulated by miRs. MDR1, a drug efflux pump overexpressed in many therapy resistant cancers, is directly regulated by miR-145 and miR-27a. Forced overexpression of these miRs reduces MDR1 protein expression in multi-drug-resistant cancer cell lines and renders them sensitive to MDR1 substrates such as doxorubicin and paclitaxel (Kovalchuk *et al*. 2008, Zhu *et al*. 2008). ABCG2 is similarly regulated by miR-328 and miR-519c (To *et al*. 2008, Pan *et al*. 2009). Re-introduction of these miRs into cancers with a multi-drug resistant phenotype holds potential as an adjuvant therapy that could potentially sensitize these cancers to chemotherapeutic agents.

As described above, the let-7 family of miRs plays a role in a host of cellular functions, and that includes modulation of drug sensitivity. The most direct mechanistic link between a let-7 family member and drug sensitivity involves let-7a, which has been shown to directly target caspase-3. Let-7a, which is overexpressed in some human cancers, induces resistance to a variety of drugs that induce caspase-3-dependent apoptosis (Tsang & Kwok 2008). Interestingly, four independent studies have found a correlation between let-7i expression and either sensitivity or resistance to certain compounds. A study on chemotherapy-resistant ovarian cancer patients determined that let-7i is down-regulated in resistant cancers, and that reintroduction of let-7i can sensitize resistant ovarian cancer cell lines to platinum-based chemotherapy (Yang *et al*. 2008*b*). A second study determined that introducing let-7i into A549 lung cancer cells sensitized them to one of ten novel chemotherapeutic compounds tested but had no effect on the others (Blower *et al*. 2008). Introducing miR-21, on the other hand, altered the sensitivity of this cell line to six of ten compounds tested. This group, however, did not test additional let-7 family members to determine whether the effect is specific to let-7i. Two studies that performed miR array analysis using either adriamycin or fulvestrant resistant derivatives of MCF7 breast cancer cells came to differing conclusions concerning let-7i. Let-7i was up-regulated in the adriamycin resistant derivative, while it was down-regulated in the fulvestrant resistant line (Chen *et al*. 2009, Xin *et al*. 2009). It is important to note that neither of these studies determined whether let-7i expression was mechanistically linked to the observed drug resistance.

Let-7 family members have also been shown to be involved in radiation response of cancer cell lines. When A549 lung cancer cells were exposed to ionizing radiation, the expression of all let-7 family members decreased except for let-7g, whose expression increased in response to radiation. Furthermore, introduction of exogenous let-7b radiosensitized these cells, while introduction of let-7g conferred radioprotection (Weidhaas *et al*. 2007). On the other hand, a similar study using PC3 prostate cancer cells found no change in any let-7 family members following exposure to ionizing radiation (Josson *et al*. 2008). A third study determined that all let-7 family members were up-regulated in Jurkat T-cell leukemia cells in response to ionizing radiation, with let-7f and let-7i up-regulated more than fourfold in these cells. Conversely, TK6 human B-lymphoblast cells sharply downregulated let-7d and let-7g in response to irradiation, while modestly upregulating the other family members (Chaudhry 2009). This study did not investigate whether exogenously altering let-7 expression affected response of the cells to ionizing radiation. In summary, it appears as if the let-7 family does play a role in the response to ionizing radiation, but that the role of this involvement may be tissue or cell type specific, potentially depending upon how much let-7 is expressed in a particular cell type.

Cell lines chronically cultured in escalating amounts of chemotherapeutic agents are a common tool used to study mechanisms of drug resistance. One study determined miR profiles in cisplatin resistant

(A2780CIS) and Taxane resistant (A2780TAX, A2780TC1, A2780TC3) A2780 ovarian cancer cells. The TC1 and TC3 cells had been derived in the presence of cyclosporine (an inhibitor of MDR1) as well as Taxol, and were 10- and 17-fold more resistant to Taxol respectively. A2780TAX cells were resistant to Taxol due to overexpression of the drug efflux pump MDR1, while the resistance of TC1 and TC3 was determined to be the result of upregulation of the class III isoform of β-tubulin, to which Taxol cannot efficiently bind. Only the A2780TAX cell line had an alteration in let-7 levels, with let-7e being up-regulated in this cell line. Interestingly, the TC1 and TC3 cell lines, despite being at least tenfold more resistant to Taxol than the A2780TAX cells, did not upregulate let-7e (Sorrentino *et al*. 2008). If let-7e upregulation could be linked mechanistically to Taxol resistance in the A2780TAX cells, it would suggest a distinct regulation of drug resistance for this particular let-7 family member that is not applicable to all forms of Taxol resistance. Interestingly, an independent study that correlated miR expression profiles of 16 ovarian cancer cell lines (including A2780 and A2780CP, a cisplatin resistant derivative) with sensitivity to six different chemotherapeutic agents (including docetaxel, doxorubicin, and cisplatin) found that let-7e was up-regulated in cell lines with increased resistance to doxorubicin (Boren *et al*. 2009). In this study, only seven miRs were significantly correlated with sensitivity or resistance to more than one compound. Of importance, none of the cell lines tested had been selected for acquired resistance to a single agent other than the A2780/A2780CP pair. This suggests that let-7e may be playing a role in both inherent and acquired resistance to particular chemotherapeutic agents. Recently, it was demonstrated that HMGA2 protects cancer cells from DNA damage-inducing reagents such as methyl methanesulphonate (Summer *et al*. 2009). However, no direct connection between let-7 and this form of drug resistance has been described.

Perhaps, the most provocative evidence linking let-7 to drug sensitivity comes from studies with breast cancer T-IC. The cancer stem cell hypothesis, while still somewhat controversial, suggests that cancers may arise from rare cells with stem-like characteristics such as self-renewal and multi-potent differentiation capability. The hypothesis further suggests that these cells are inherently drug resistant, and that chemotherapy fails in part because of the ability of these cells to survive and repopulate the tumor. Recent evidence has demonstrated that breast cancer T-ICs can be characterized by low let-7 expression, and that modulating let-7 expression in these cells alters their stem-like properties (Yu *et al*. 2007*a*). This phenomenon was also observed in comma-Dβ, an immortalized but not transformed mouse mammary epithelial cell line that contains a permanent population of undifferentiated progenitor cells that are able to repopulate the mouse mammary tree. These progenitor cells were found to be let-7 low, and enforced let-7 expression eliminated the self-renewing cells from the population (Ibarra *et al*. 2007). Taken together, these studies suggest that a let-7 low status is common to both T-ICs and normal epithelial progenitor cells.

Yu *et al*. initially observed that cells taken from breast cancer patients who had been treated with chemotherapy had greater tumorigenic properties than those obtained from chemotherapy-naïve patients. In order to investigate this phenomenon further and determine the molecular basis, the group devised a method to obtain large amounts of these T-ICs. The cells were derived by serially passaging the SKBR3 breast cancer cell line through NOD/SCID mice that had been injected with epirubicin. The resulting cells (SK3RD) were cultured in an attachment-free culture system and displayed many of the stem-like properties of the cells derived from chemotherapy-treated patients. As with the patient cells, it was determined that SK3RD cells had a much greater ability to proliferate in suspension and form ball-like structures termed mammospheres when compared to parental SKBR3 cells. The cells were highly enriched for a $CD44^{+}CD24^{-}lin^{-}$ stem cell marker phenotype, and plating them on collagen under differentiating conditions drastically reduced the number of cells with this phenotype. SK3RD cells were also determined to have significantly greater resistance to epirubicin prior to differentiation and expressed the ABC transporter ABCG2.

The group then performed miR array analysis on SK3RD cells prior to and after differentiation and found that all let-7 family members were expressed at a low level in SK3RD cells but were significantly up-regulated post-differentiation. Furthermore, they determined that introducing let-7 into these cells drastically reduced their tumorigenicity, as determined by their ability to form mammospheres as well as their ability to form serially transplantable tumor xenografts in nude mice. No further investigations were done concerning drug sensitivity, but it is interesting to suggest a hypothesis where a tumor, even one that largely maintains let-7 expression, is populated by let-7 low T-ICs that are more resistant to chemotherapeutic agents than the tumor as a whole. Successful therapy in this scenario would then be predicated on the ability to target these T-ICs. Much work remains, but this initial

insight into the relationship between let-7 and T-ICs strongly supports the growing body of evidence that let-7-based therapeutics will likely hold significant promise as both a frontline and an adjuvant treatment option for a variety of different cancers.

Outlook

As we have discussed throughout this review, the let-7 family of miRs plays a role in an exceedingly diverse array of cellular activities. The role of let-7 in the differentiation of multiple cell types across multiple organisms during embryonic development has been firmly established. Furthermore, there is a very clear link between loss of let-7 expression and the development of poorly differentiated, aggressive cancers. As we have elucidated, let-7 expression is regulated on multiple levels, and particular family members appear to be specifically deregulated in certain cancers. Questions remain, however, as to whether 13 let-7 family members exist because they have different activities (targets) or whether that many genes exist to allow a more differentiated regulation in various cell/tissue types. In addition, while the evidence is growing that loss of let-7 increases resistance to certain chemotherapeutic drugs and to radiation, it is at present unknown how this occurs mechanistically because no let-7 target has been identified that would provide an explanation of this activity of let-7. Regardless of the answers to these questions, available data suggest that restoration of let-7 expression to tumors where it has been lost holds great therapeutic potential for the treatment of these aggressive types of cancer.

Declaration of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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