Review

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The role of microRNA in nutritional control

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Abstract. Nolte-'t Hoen EN, Van Rooij E, Bushell M, Zhang C-Y, Dashwood R, James WPT, Harris C, Baltimore D (Utrecht University, Utrecht, The Netherlands; Hubrecht Institute, Koninklijke Nederlandse Academie van Wetenschappen (KNAW), University Medical Center Utrecht, Utrecht, The Netherlands; University of Leicester, Leicester, UK; Jiangsu Engineering Research Center for microRNA Biology and Biotechnology, State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing, China; Center for Epigenetics and Disease Prevention, Institute of Biosciences & Technology, Texas A&M Health Science Center, Houston, TX, USA; London School of Hygiene and Tropical Medicine, London, UK; National Cancer Institute, Center for Cancer Research, National Institutes of Health, Bethesda, MD, USA; and California Institute of Technology, Pasadena, CA, USA) The role of microRNA in nutritional control. (Review) *J Intern Med* 2015; **278**: 99–109.

MicroRNAs (miRNAs) are one of a growing class of noncoding RNAs that are involved in the regulation of a wide range of metabolic processes including cellular differentiation, cell proliferation and apoptosis. The generation of miRNA is regulated in complex ways, for example by small interfering RNAs (small nucleolar and nuclear RNAs) and various other metabolites. This complexity of control is likely to explain how a relatively small part of the DNA that codes for proteins has enabled the evolution of such complex organisms as mammals. Non-protein-coding DNA is therefore thought to carry the memory of early evolutionary steps that led to progressively complex metabolic controls. Clinically, miRNAs are becoming increasingly important following the recognition that some congenital abnormalities can be traced to defects in miRNA processing. The potential for manipulating metabolism and affecting disease processes by the pharmaceutical or biological targeting of specific miRNA pathways is now being tested. miRNAs are also released into the extracellular milieu after packaging by cells into nano-sized extracellular vesicles. Such vesicles can be taken up by adjacent and possibly more distant cells, thereby allowing coordinated intercellular communication in specific tissues. Extracellular miRNAs found in the blood stream may also serve as novel biomarkers for both diagnosing specific forms of cancer and assessing the likelihood of metastasis, and as powerful prognostic indices for various cancers. Here, we discuss the role of intracellular and extracellular miRNAs in nutritional control of various (patho)physiological processes. In this review, we provide an update of the presentations from the 25th Marabou Symposium (Stockholm, 14–16 June 2013) entitled 'Role of miRNA in health and nutrition', attended by 50 international experts.

Keywords: development, metabolic control, miRNA, nutrition, pathology, signalling.

FOR A COMPILATION OF THE GENERAL DISCUSSION AT THE MARABOU SYMPOSIUM, click here http://www.marabousymposium.org/

Introduction

MicroRNAs (miRNAs) play fundamental roles in the control of development and metabolism. Ongoing research of these genetic components allows a new understanding of mechanisms by which nutritional factors can control development. miRNAs belong to a large group of noncoding RNAs with gene-regulatory properties. The proportion of non-protein-coding DNA involved in regulation of protein synthesis increases with developmental complexity, whereas the number and repertoire of

protein-coding genes remain relatively static. In prokaryotes, 5–25% of the total DNA is nonprotein coding, whereas the non-protein-coding DNA of eukaryotes increases from 50% in simple eukaryotes to 75% in plants and invertebrates and to 85–95% in higher vertebrates [1]. Furthermore, it is now recognized that the proportion of DNA transcribed into RNA in higher organisms is far higher than previously considered. The total mammalian transcriptome is an extraordinarily complex network of regulatory molecules and harbours a large variety of small and long non-protein-coding RNAs, including miRNAs, small interfering RNAs (siRNAs), small nucleolar RNAs (snoRNAs) and small nuclear RNAs (snRNAs). This regulatory power of noncoding RNA seems to underlie human evolution, development and even such complex processes as cognition [2] and also affects the pathogenesis of a variety of diseases.

The generation of miRNAs and the complex feedback controls

The miRNAs are initially transcribed from DNA in a precursor form as primary miRNAs (pri-miRNAs), which are themselves tightly regulated (Fig. 1). Many DNA segments that code for miRNAs are clustered tightly together. Expression profiles within a cluster are very similar, with new evidence suggesting that the synthesis of clusters is controlled as a package by a single promoter, leading to the generation of a pri-miRNA that in turn may contain one to six or more precursor microRNAs (pre-miRNAs). At least one-third of human miRNA genes are clustered in this fashion. Conservation of miRNA clusters across species suggests that evolutionary pressure has maintained this organized structure. The production of individual pre-miRNAs from pri-miRNAs requires an additional step. The pri-miRNAs are bound within the nucleus to a DiGeorge syndrome critical region 8 (DGCR8) protein that facilitates the cleavage of the pri-miRNA by a ribonuclease III protein, that is DroshaRNase (Fig. 1). This cleavage results in the production of double-stranded pre-miRNAs. These precursors are exported to the cytoplasm (facilitated by RAN GTPase and Exportin 5) where loops found at the end of the double-stranded molecule are severed by a protein complex (DICER 1, EIF2C1, EIF2C2, GEMIN3, GEMIN4 and TRBP). This second cleavage results in a double-stranded RNA containing about 22 nucleotides (Fig. 1) [3]. Then, one strand (the miRNA) is loaded onto a protein complex, the RNA-induced silencing complex (RISC), in a very precise way so that the hydrogen bonding potential of the bases is open. In the RISC complex, miRNAs can find complementarity with the three-prime untranslated regions (3′UTRs) of messenger RNAs (mRNAs). The interaction involves six to eight nucleotides at the five-prime end of the target mRNA. This short 'seed' sequence of nucleotides allows great specificity in the binding of miRNA to targeted mRNAs. This complex silences the expression of target genes predominantly at the post-transcriptional level. When miRNAs direct their targets to the mRNA decay pathway, the mRNAs are first deadenylated, for example by the recruitment of deadenylases by a protein of the GW182 family. In addition, miRNAs can mediate active repression of translation. The relative contributions of translational repression and mRNA destabilization to miRNA-mediated control of gene expression were long unclear. However, recent data indicate that translational control by miRNA is a primary event and that this is a prerequisite for target mRNA degradation to occur [4].

miRNA also has a promiscuous dimension in that specific miRNAs may interact with a variety of mRNAs that encode different proteins. This allows a single miRNA to influence a set of genes in a shared pathway or a protein complex. The specificity of the seed sequence and its ability to target an overlapping set of gene products allow the grouping of miRNA into different 'miRNA families' with different specificities of action. Given the multiple processing steps in the generation of miRNAs, there are many points at which its production can be controlled. It is now also apparent that a variety of metabolites and other signals, such as the cytokine transforming growth factor β, can affect miRNA generation and therefore the transcription and translation of several genes. Importantly, evidence is accumulating that many miRNAs can be deleted without creating a clear phenotype. This could be explained by functional redundancy of many miRNAs that share the same seed sequence. An alternative explanation is that the main function of miRNAs is to balance variations in gene expression levels, causing miRNA phenotypes to occur only upon stress.

Intercellular signalling

It has recently become clear that miRNAs can also transmit regulatory signals between cells. Intercellular communication via miRNA-containing vesicles is now recognized as an important cellular strategy to convey messages to adjacent or more

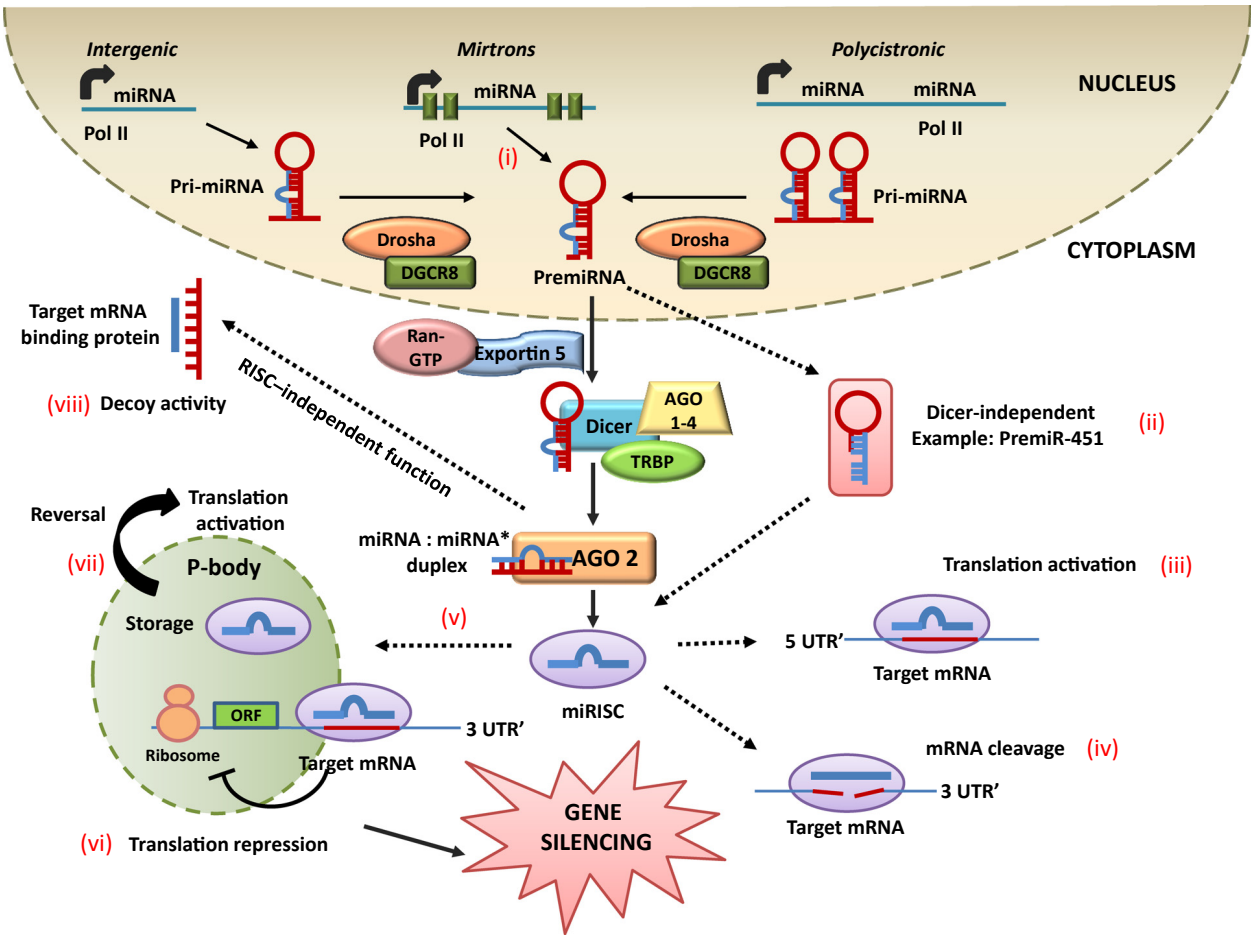

Fig. 1 *MicroRNA (miRNA) biogenesis and regulatory pathways. Primary miRNAs (pri-miRNAs) are transcribed from RNA polymerase II-specific miRNA genes, from the intronic region of protein-coding genes, or from polycistronic transcripts. In the first nuclear step, pri-miRNA is processed into a 70- to 100-nucleotide precursor hairpin (pre-miRNA) via the Drosha–DGCR8 complex. Pre-miRNA is transported to the cytoplasm through export machinery consisting of Exportin 5 and Ran-GTP. Here, the pre-miRNA is cleaved by another endoribonuclease, Dicer, in partnership with TRBP and Ago proteins, forming a 20-bp miRNA: miRNA* duplex. After processing, one strand of the duplex is preferentially incorporated with the help of Ago2 into the RISC complex (miRISC), whereas the other 'passenger' strand (miRNA*) is degraded. (i) A few pre-miRNAs are processed directly from short introns (mirtrons), bypassing the Drosha–DGCR8 step. (ii) In a Dicer-independent mechanism, miRNA is cleaved by Ago2 to form a mature miRNA. (iii) Some miRNAs bind to the 5′UTR of the target messenger RNA (mRNA) and lead to translational activation. (iv) Full or near-full complementarity between miRNA and mRNA target facilitates miRISC-directed cleavage of the mRNA target. (v) With low complementarity, miRNA-mediated regulation is carried out by translational repression. (vi) This can occur pre- and/or postinitiation of translation leading to gene silencing. (vii) Target mRNAs also can be stored in P-bodies, and the mechanism reversed by re-entry into polysomes for translation. (viii) In an RISC-independent decoy activity, miRNAs can directly bind to proteins, particularly RNA-binding proteins, making them unavailable for binding to their RNA targets. From Parasramka* et al. *2012 [3], with permission.*

distant cells [5]. Such extracellular vesicles can be derived from different subcellular compartments (Fig. 2). Exosomes are released after fusion of late endosomal compartments (multivesicular bodies) with the plasma membrane, whereas microvesicles are formed by direct budding from the plasma membrane itself. This is an active process whereby the incorporation of lipids, proteins and RNAs into the vesicles is regulated strictly by the producing cell. A prominent focus within the research of extracellular vesicles is to uncover their role in innate and adaptive immune responses. For example, it has been demonstrated that several activation signals regulate the composition, release and

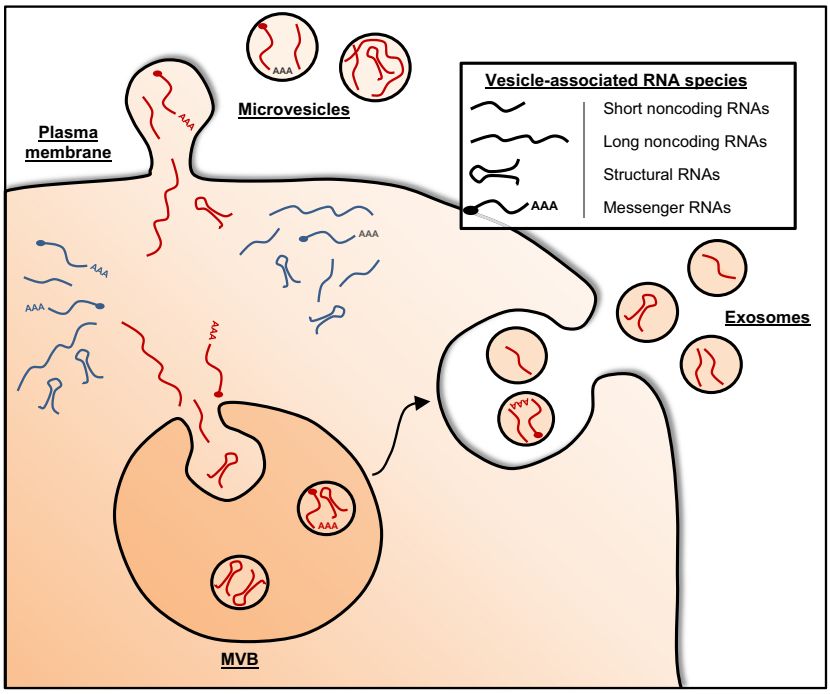

Fig. 2 *Formation of RNA-containing extracellular vesicles. In mammalian cells, subpopulations of extracellular vesicles arise in different subcellular compartments. Exosomes arise in the endosomal system and are released upon fusion of multivesicular bodies (MVBs) with the plasma membrane. Alternatively, microvesicles can directly pinch off from the plasma membrane. Extracellular vesicle-associated RNA species include short noncoding RNAs (e.g. microRNA and tRNA fragments), long noncoding RNAs, structural RNAs (e.g. vault RNA and signal recognition particle RNA (SRP-RNA)) and protein-coding messenger RNAs. Adapted from van der Grein and Nolte-'t Hoen [10].*

targeting of vesicles released by dendritic and T cells [6, 7]. Analysis of these vesicles requires dedicated nanotechnology, such as the newly developed high-resolution flow cytometric technique for high-throughput multiparameter analysis of individual nano-sized vesicles [8]. RNA deep sequencing studies of vesicles derived from diverse cell types indicate that these vesicles contain not only specific miRNAs, but also a variety of other small and long noncoding RNAs with potential gene-regulatory activity [9] and message mRNAs [9, 10] (Fig. 2). Horizontal vesicle-mediated transfer of RNA uniquely allows the intercellular dissemination of genetically encoded messages, which may modify the function of target cells.

miRNAs and development

It is recognized that foetal development involves multiple miRNA-mediated mechanisms that play a critical part in the programming of cellular and tissue organization. These developmental processes require precisely timed and coordinated activation and inactivation of cell division, cellular differentiation and cellular interactions. Finely controlled stem cell activity often underlies this developmental sequence. With the current identification of over 2500 miRNAs, the emerging view is that various clusters of miRNAs can induce or repress similar cellular processes in multiple tissues. As an example, miRNAs influence stem cell activity in cardiac, renal, gut, adipose, brain, skeletal, skin and immune tissues by providing a complex feedback system to preserve the stability of gene expression (see below). By contrast, genetic abnormalities in miRNA gene clusters can lead to severe pathologies and may even be (maternally) inherited [11].

In immune development and function as well as in haematopoiesis, miRNAs also play a role in controlling cell development and in maintaining the stability of cellular function. Various miRNAs are known to control the developmental steps that lead to differentiation of bone marrow stem cells into the broad range of lymphoid and myeloid cells. In this

process, miRNAs often induce transcription factors that in turn may suppress the synthesis of an opposing miRNA or even of itself through a negative feedback mechanism [12]. Besides their role in immune cell development, miRNAs also regulate the function of these cells. miR-146a, for example, prevents the unbridled activation of nuclear factor-kappa B (NF-kB), which is a key transcription factor in inflammatory and other immune responses [13]. By limiting the response to pathogen-associated molecular patterns, miR-146a guards against pathogenic immune stimulation. This suppression of NF-kB is a crucial function as the miR-146a knockout mouse develops bone marrow aplasia after 8 months with the later development of myeloid cancer [14]. During chronic inflammation, this miRNA is also an important regulator of the quality and longevity of haematopoietic stem cells.

Nutritional control of miRNA function

Mammalian foetal development is dependent on an adequate placental supply of multiple substrates, including classic nutrients and hormones. It is well recognized that differences in the availability of nutrients not only alter foetal growth but also induce permanent changes in the metabolic responsiveness of the developing foetus. The mechanisms underlying these nutritional effects have long been unclear. A number of miRNAs have now been identified that are susceptible to regulation by maternal diet. One of these is miR-483-3p, which is located in an intron of the *Igf2* gene. This miRNA controls the ability of adipose tissue to store lipid and has a direct effect on growth differentiation factor 3 [15]. Increased miR-483 expression *in vivo*, programmed by early-life nutrition, limits storage of lipids in adipose tissue and thereby may be linked to insulin resistance and an increased susceptibility to metabolic diseases such as type 2 diabetes. In line with this, the miRNA-mediated regulation of the retinoid x receptor RXR may also play an important role in childhood adiposity. The RXR is involved in the control of insulin sensitivity, adipogenesis and fat metabolism. Studies of early human pregnancy demonstrate that the effects of a high-fat, low-carbohydrate diet are not only strongly related to the methylation of the RXR receptor in the foetal tissue, but that these changes are also strongly associated with the child's adiposity at 9 years of age [16, 17]. As miRNAs control both the expression of the RXR receptor [18] and its promoter methylation [19], nutritional effects on RXR receptor-controlled development of adiposity appear to be, at least partly, mediated by miRNAs.

miRNAs in human milk

Particularly intriguing from a nutritional point of view is the discovery that human milk contains extracellular vesicles with miRNAs that may play a role in the contribution of human milk to instructing the infant immune system [20]. As many as 1081 miRNAs were found in the lipid fraction of (postcolostrum) human milk and 9074 mRNA targets were identified for these miRNAs [21]. In addition, the secretion of miRNAs in human milk responds to changes in maternal diet, especially dietary fat. This may be a means by which maternal diet influences metabolism and responsiveness of the infant immediately after birth when the intestine appears to be transiently permeable to complex molecules. Although several lines of evidence indicate that miRNAs in mother's milk could affect both the development and the metabolic responses of young animals or children, it is currently unknown where miRNA-containing vesicles are absorbed in the gastrointestinal tract and how they exert their regulatory function.

Are metabolically active miRNAs absorbed in adult life?

The mammalian intestine reaches a stage during development at which it becomes less permeable to complex molecules; however, new evidence suggests that ingested miRNAs (e.g. from plants) may be absorbed even in adult life and exert specific metabolic effects. Although this concept remains highly controversial [22, 23], Zhang and colleagues have identified a variety of plant miRNAs in the blood of rodents, calves and humans [24]. The authors described plant miRNAs in circulating microvesicles. The detected presence of plant miRNAs appears to depend on the type of diet fed to experimental animals, with clearly increased plasma levels being found a few hours after a meal. Some of these miRNAs were reported to affect gene expression in mammalian tissues. For example, rice-derived miR-168a binds to exon 4 of the mammalian low-density lipoprotein receptor adaptor 1 (*LDLRAP1*) gene in the liver. This binding was found to reduce the production of the LDL receptor protein, which is responsible for the removal of LDL cholesterol from blood [24]. Increased plasma LDL levels were reported in mice 3–7 days after feeding mice rice. These findings could indicate a new aspect of the nutritional control of metabolism [25].

Role in metabolism and nutritional responses

The results of numerous studies indicate that miRNAs control metabolism and that miRNA levels change in response both to diet and to subsequent changes in nutritional state (Table 1). Moreover, it is becoming apparent which component of the metabolic pathways in any regulatory mechanism is regulated by a particular miRNA (Table 2). miR-122, for example, has been implicated in the biosynthesis, metabolism and transport of cholesterol [26] with the cholesterol transporter for high-density lipoprotein levels being modulated by miR-33 which also affects fatty acid synthesis and plasma triglyceride levels [27]. miRNAs also participate in the regulation of glucose metabolism [28] by modifying the activity of caveolin-1, a critical regulator of the insulin receptor, whereas miR-143, which is overexpressed in obesity, impairs insulin-stimulated AKT protein kinase activity, thereby also affecting glucose homoeostasis and insulin resistance [29]. Let-7, which is normally associated with the regulation of oncogenes, has recently been found to be involved also in multiple pathways affecting insulin sensitivity [30]. Obesity is known to impair insulin sensitivity and acts by inducing the hepatic overexpression of miR-802, which silences hepatocyte nuclear factor 1 homeobox B (Hnf1b) activity and then leads to glucose intolerance, impaired insulin signalling and promotion of hepatic gluconeogenesis [31]. Other miRNAs (e.g. miR-375) affect the maintenance of both the α and β cells of the pancreas [32], whereas the capacity of the pancreatic β-cell mass to induce insulin exocytosis is affected by miR-7a; the miR-7a levels were found to be decreased in obese/diabetic mouse models and human islets from obese and moderately diabetic individuals with compensated β-cell function [33]. miR-204, however, blocks insulin production by directly targeting and downregulating MAFA, a known insulin transcription factor [34]. With the renewed interest in the control of the development and modulation of brown adipose tissue (BAT) in relation to obesity in both animals and man [35], miR-133 and the cluster miR-193b-365 are now seen as potentially important controllers of BAT development [36, 37] and the response of this tissue to cold. The miR-155 is also involved in regulating the commitment of brown adipocytes [38]. Another miRNA shown to be involved in the induction of obesity by high-fat feeding of animals is the heart-specific miR-208 [39]. This miRNA governs energy homoeostasis by affecting MED 13, a subunit of the Mediator complex that controls the transcription of thyroid hormone and other nuclear hormone receptors. Changes in cardiac-specific MED13 influence the fat mass of animals by altering general energy expenditure without any effects on food intake or physical activity and without inducing any changes in the natriuretic hormone responses. These findings imply that

Table 1 *Examples of miRNAs implicated in major metabolic processes of clinical significance*

miRNA	Role in metabolism	Reference
miR-122	Biosynthesis, metabolism and transport of cholesterol	[26]
miR-33	Cholesterol transporter for HDL; fatty acid synthesis	[27]
miR-103 and miR-107	Insulin resistance by altering the sensitivity of the insulin receptor	[28]
miR-143	Impairment of insulin-stimulated AKT activation and glucose homoeostasis with insulin resistance	[29]
Let-7	Insulin resistance	[30]
miR-802	Obesity-induced hepatic gluconeogenesis and glucose intolerance	[31]
miR-375	Maintenance of α- and β-cell mass	[32]
miR-200	β-cell survival	M. Stoffel, Unpublished data
miR-7	Insulin secretion; β-cell dedifferentiation	[33]
miR-204	Insulin secretion	[34]
miR-133	Brown and brite fat cell function	[36]
miR-193b-365	Brown fat differentiation	[37]
miR-155	Regulation of the commitment to differentiation of brown and beige adipocytes	[38]
miR-208	Heart-specific miR affecting energy homoeostasis	[39]

Table 2 *The targeted genes or pathways involved in the regulation of metabolism by some miRNAs*

miRNA	Targeted genes or pathway
miR-122	Glucose metabolism; cholesterol biosynthesis
miR-33	Cholesterol efflux (AbcA1); fatty acid oxidation
miR-103	Regulation of insulin receptor signalling and Cav1
miR-143	Possible regulation of insulin sensitivity through ORP8, a protein involved in AKT activation Let-7, insulin/PI3K signalling
miR-802	Regulation of Tcf2, transcriptional control of insulin signalling in liver
miR-375	Regulation of negative growth regulators (Rasd1, HuD, Rgs16, Dadm1, Eef1e1)
miR-200	Unknown
miR-7	Regulation of *NeuroD1* and *Gata6,* and of insulin granule trafficking and fusion
miR-204	Regulation of insulin transcription via TXNIP and MafA
miR-133	Prdm16 transcriptional network
miR-208	THRAP1, also known as TRAP240, component of the thyroid hormone receptor (TR)-associated TRAP complex, modulates activity of the TR by recruitment of RNA polymerase II and general initiation factors
miR-193b-365	Regulates *Runx1t1*, represses myogenesis and enhances brown adipocyte differentiation
miR-155	Integration of hormonal signals that regulate proliferation or differentiation of preadipocytes through adipogenic transcription factor CCAAT/enhancer-binding protein β

Cav1, caveolin-1; ORP8, oxysterol-binding protein-related protein 8; AKT, protein kinas B; PI3K, phosphoinositide 3-kinase; tcf-2, transcription factor 2; TXNIP, thioredoxin-interacting protein; TRAP, translocon-associated protein; CCAAT box, distinct pattern of nucleotides with GGCCAATCT consensus sequence.

there is a secreted cardiac signal that alters tissue metabolism and could be operating through the generic Mediator complex in different tissues.

A novel class of chemically engineered oligonucleotides, termed 'antagomirs', are efficient and specific silencers of endogenous miRNAs. Intravenous administration of antagomirs against miR-16, miR-122, miR-192 and miR-194 in mice resulted in a marked reduction in the corresponding miRNA levels in the liver, lung, kidney, heart, intestine, fat, skin, bone marrow, muscle, ovaries and adrenal glands [26]. Antagomirs have also been used to silence miR-103 and miR-107, which are upregulated in the liver of obese mice and humans with hepatic steatosis [28]. In animal studies, the silencing of endogenous miRNAs by this novel method seems to be specific, efficient and long-lasting. Antagomir-induced silencing of miR-103/miR-107 led to a reduction in the overall fat mass and adipocyte size in obese mice and to an increase in adipocyte glucose uptake without enhancing insulin signalling [28]. By contrast, antagonising miR-7a2 improved insulin secretion and glucose tolerance, whereas overexpression of this miRNA led to dedifferentiation of pancreatic β cells [33]. The above examples illustrate how the pharmaceutical or biological targeting of specific miRNA pathways in animal studies may lead to strategies to manipulate metabolism and disease processes in humans.

miRNAs in carcinogenesis and miRNA-based monitoring of disease progression

Several lines of evidence indicate that diet impacts cancer rates in both animals and man [40]. Food components can either promote or repress carcinogenesis and some of these effects involve miRNAs [3].For example, colon cancer can be experimentally induced with known mutagenic heterocyclic amines that are generated during the cooking of meat and fish [e.g. 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP)] and miR-206 is highly upregulated in this process [41]. The let-7/c-Myc/Lin28 axis is also dysregulated in PhIP-induced rat colon carcinogenesis, and feeding spinach to exposed animals not only inhibited tumour formation but also partially normalized this pathway [42]. Nutritional factors may also affect cancer rates indirectly, for instance by inducing obesity and the consequent inflammatory effects.

The importance of miRNA not only in modulating metabolism but also in demonstrating the propensity to cellular damage and cumulative genetic change is becoming apparent with the emerging

evidence that analyses of tissue miRNA can help both to reveal the presence of a particular cancer type and to assess its capacity for metastasis and progression. This can also lead to new insights into therapeutic targets. The familial propensity for different cancers can also now be assessed by analysing single nucleotide polymorphisms in pri-miRNA, pre-miRNA or the seed regions of miRNAs, implying that these genetic distinctions operate at least in part through heritable differences in the noncoding RNAs. However, care needs to be taken in generalizing such findings because, for example, robust distinctions in chromosomal miRNA profiles related to differential risk of lung cancer may be evident in both European American and Japanese populations, but not in African Americans [43]. Monitoring the miRNA profiles of primary tumours may also assist in determining patient prognosis. For example, significant differences can be found between the miRNAs of primary lung cancers and corresponding noncancerous lung tissues and between histologically distinct types of lung cancer [44]. Increased miR-21, miR-155 and miR-106b, as well as decreased let-7, are all associated with the diagnosis and prognosis of lung cancer [45]. Some solid tumours also share particular miRNA patterns [42]. For instance, miR-21 was found to be upregulated in tissues obtained from 18 major cancers and is a powerful prognostic biomarker of more rapid progression in 10 cancer types [43–56] as shown in Fig 3. The combination of protein-coding and noncoding gene expression was also found to be a robust prognostic classifier in stage I lung adenocarcinoma [57]. Recent findings indicate that, in addition to their presence in tumour tissue, miRNAs are also found extracellularly in body fluids, such as blood and urine, and can serve as a novel class of biomarkers for cancer diagnosis and progression [58]. These miRNAs can be associated with extracellular vesicles or enclosed in nucleoprotein complexes, and their presence and abundance in body fluids can reflect the health or disease status of the tissues from which they derive. For both the tumour cell-associated

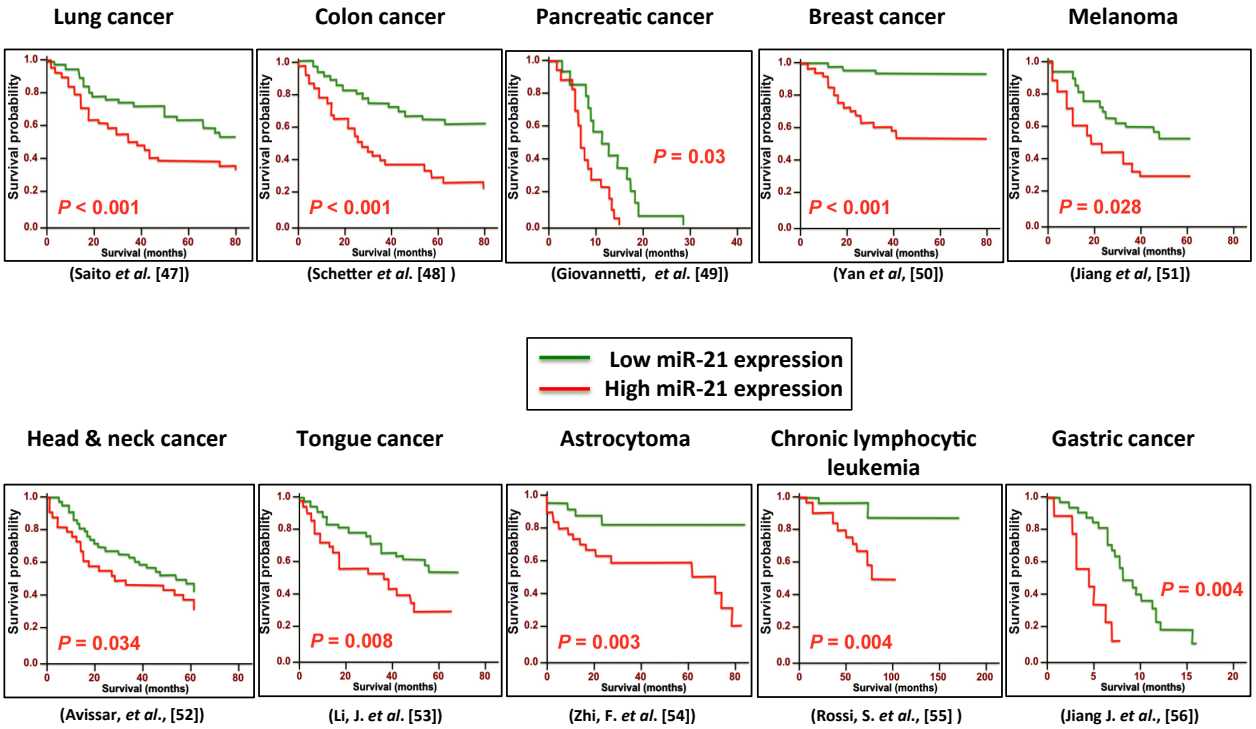

Fig. 3 *Overview of cancer types in which expression of the microRNA miR-21 in tumour tissue is a powerful prognostic biomarker of the subsequent more rapid progression of each cancer. Kaplan–Meier curves show differences in survival between patients with high (red lines) or low (green lines) miR-21 expression (as defined in the indicated original publications).*

miRNAs and extracellular miRNAs, it is important to assess how robust correlations with disease activity are in several similar and diverse (patient) cohorts.

Conclusions

The focus on miRNAs as major controllers of development, cell stability and metabolic regulation is greatly increasing our understanding of factors governing optimal foetal growth and development and of the effects that nutritional factors have on these processes. In addition, the results from miRNA studies highlight the mechanisms underlying the evolution of different tissue structures, and their functioning and susceptibility to disease. miRNAs exert their effects not only on intracellular processes, but also during intercellular communication upon their release in extracellular vesicles or nucleoprotein complexes and uptake by neighbouring cells. Greater understanding of miRNA biology is starting to explain the coordinated activity of different tissues within different nutritional and toxic environments. Moreover, these studies have revealed potential therapeutic targets for treating a variety of disorders including obesity, hepatic steatosis, diabetes mellitus, cardiovascular disease, mental disorders and cancer. Thus, the plethora of cellular mechanisms involving miRNAs and their susceptibility to nutritional influences present a series of promising research challenges in this rapidly evolving field.

Conflict of interest statement

Drs Dashwood, Bushell, James, and Harris have nothing to disclose. Dr Nolte-'t Hoen reports a grant (11676) from a partnership programme jointly funded by Nutricia Research and the Dutch Technology Foundation STW, which is part of the Netherlands Organization for Scientific Research (NWO), and is partly funded by the Ministry of Economic Affairs, during the conduct of the study. Dr Zhang reports grants from Ministry of Science and Technology of the People's Republic of China, grants from National Natural Science Foundation of China, during the conduct of the study. Dr van Rooij reports personal fees from miRagen Therapeutics, related to the subject matter and has several issued patents. Dr Baltimore reports personal fees from Regulus Therapeutics as a board member, outside the submitted work.

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