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Non-coding RNAs – A primer for the laboratory scientist

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Abstract

Advances in molecular genetics have identified several species of RNA that fail to translate – hence the non-coding RNAs. The two major groups within this class of nucleic acids are microRNAs (miRNA) and long non-coding RNAs (lncRNA). There is growing body of evidence supporting the view that these molecules have regulatory effect on both DNA and RNA. The objective of this brief review is to explain the molecular genetic of these molecules, to summarise their potential as mediators of disease, and to highlight their value as diagnostic markers and as tools in disease management.

Introduction

The human genome of nuclear and mitochondrial DNA is believed to contain some 6.4 billion nucleotides arranged as 3.2 billion base pairs, many of which are arranges as genes. Some 70-90% of the genome is transcribed, the remainder being introns (intervening sections between functioning genes), and which include some 14,000 pseudogenes (genes that have become non-functioning, probably because of inconsequential change in the DNA) that comprise between 2% and 10% of the genome. Of the genomic DNA that is transcribed, 60% is processed into viable, functional RNA, and includes 20,000 genes that will go on to code for proteins (~2% of the genome) and around 22,000 genes that do not go on to code for protein (i.e. are non-coding RNAs). Mitochondrial DNA contrasts with nuclear DNA in that almost 93% is coding, and there are no introns. The non-coding RNAs include transfer RNA (10-15% of total RNA), ribosomal RNA (80-90%), circular RNA, small-interfering RNA, small nuclear RNA, PIWI-interacting RNA (26-31 nucleotides, PIWI being a protein with regulatory activity in stem cell differentiation), and small nucleolar RNA (all <1%), and there are some 15,000 pseudogenes (comprising 2 – 10% of the genome). Two other groups are recognised and can be classified by their size into those of around 22 nucleotides in length, and those of over 200 nucleotides: respectively, microRNAs (miRNAs, 0.003 – 0.02% of total RNA) and long-non coding RNAs (lncRNAs, 0.06 – 0.2%)(1-4). Both have roles in physiology and pathology, and their detection in blood and tissues, using techniques in molecular genetics, allows scientists to develop them as

potential tools as biomarkers in the diagnosis of a diverse group of diseases (6-8). Furthermore, in several cases interacting pharmaceuticals can be developed, and these may have a value in treatment.

The objective of this brief review is to summarise the potential of microRNAs and long-non-coding RNAs as mediators of disease, and to highlight their value as diagnostic markers of disease and as tools in disease management.

MicroRNAs

miRNAs are small regulatory sections of RNA coded for by nuclear DNA and transcribed by RNA polymerase II. With a few exceptions, each miRNA is named by miR- followed by a designated number, their function being to silence their complementary mRNA, either by RNA cleavage or by suppression of translation. Indeed, it has been suggested that miRNAs target a third of all human genes, and there are instances where a miRNA increases the expression of its target mRNA, and some where the target can be DNA (as in the case of miR-373, coded for at 19q13.4)(9). The concept of RNA inhibiting other RNA can be traced back to the 1980, with the production of synthetic antisense RNAs with biological activity (10). However, perhaps the earliest demonstration of an miRNA was lin-14 in the nematode worm *Caenorhabditis elegans* in 1993, and in our own species in 2002 where they are now estimated to regulate somewhere between a third and 60% of genes in the genome (11,12).

The initial synthesis of an miRNA transcript by a polymerase generates a pri-miRNA molecule which in secondary structure has a hairpin shape. 5' and 3' tails are trimmed within the nucleus by Drosha, a ribonuclease III, yielding a ~60 nucleotide pre-miRNA that translocates to

the cytoplasm and binds with Dicer, an endonuclease that dissociates the secondary structure. One of the single stands, in conjunction with a protein called Argonaute, forms an RNA-induced silencing complex (RISC). The single strand miRNA binds to is target mRNA within the RISC, resulting in the degradation of the message (5,6,12). Genes coding for miRNAs may be found by themselves, or in polycistron clusters that together may contain >33% of the total miRNA pool (13). One cluster, that of miR-17-92 at 13q31-q3, produces seven mature miRNAs: miR-17-3p, miR-17-5p, miR-18a, miR-19a, miR-19b, miR-20a, and 92a. More than 30 downstream targets of these miRNAs have been reported in diverse conditions – stroke, heart disease, bone development and in cancer. The ever-expanding field of miRNA forces us to focus on only a few areas of pathology.

miRNAs in haemopoiesis

Several miRNAs – miR-29a, miR-126, miR-155 and miR-125a/b – are expressed in haemopoietic stem cells and over-expression or inhibition can influence stem cell biology, whilst miR-23 may part-regulate erythropoiesis (3,14,15). Indeed, as other miRNAs, principally miR-221/222 and miR-223, are involved in erythroid differentiation, there is potential for these molecules to be engineered into mimics that will stimulate red blood cell production in certain anaemias (16). An example of this may be the manipulation of transcription factor *MYB* by miR-15a and miR-16-1 in elevating foetal haemoglobin levels and so hopefully transfer this technology to sickle cell disease and β-thalassaemia. (17). Multiple miRNAs, including miR-17-92, miR-34a, miR-150 and miR-181, regulate early B lymphocyte differentiation, whilst

expression of miR-125b directs differentiation away from the B-cell lineage and towards the myeloid lineage (18,19).

Given the role of miRNAs in haemopoiesis, it is perhaps not surprising to find that they have roles in leukocyte malignancies (7,19,20). One of the first reports of miRNA in cancer was in chronic lymphocytic leukaemia (CLL)(21). miR-15 and miR16 are coded at 13q14, and are deleted or down-regulated in over two-thirds of cases of CLL. Their expression inversely correlates with the expression of *BCL-2*, a mitochondrial protein with roles in apoptosis. Further links between miRNAs and CLL are demonstrated by the report that of a deregulated miR expression pattern (miR-34a, miR-29 and miR-17-5p) in patients with deleted and/or mutated tumour suppressor *p53* (22). Silencing miR-330-5p in vitro down regulates markers of leukaemogenesis (23), whilst epigenetic methylation of tumour suppressor miR-340-5p is a likely early event in myelomagenesis (24). Observations of this nature prompt the possibility of using miRNAs in a clinical setting as therapeutics. miR-650 in CLL provides a further case model, being coded for by a gene close to the immunoglobulin kappa light chain (IgLλ) gene (25). One suggestion for a relationship between the two is that the IgLλ promotor is an enhancer element for miR-650. The link with transformation is that miR-650 reduces the expression of CKD1, a key component of the control of the cell cycle checkpoint at G_1/S. Notably, CLL patients with higher expression of miR-650 had improved survival and a longer time to first treatment, suggesting clinical relevance and a role in management.

miRNAs in solid tissue cancer

The list of miRNAs with likely roles in cancer grows exponentially, and in this disease many (such as those of the let-7 family) act to inhibit tumour suppressors (such as *p53*, *MYC,* and *Rb*, often regulators of the cell cycle), whilst others (such as the miR-17-92 cluster and miR-155) may also act as oncogenes in their own right, and some (such as miR-165 and miR-166) have epigenetic effects (6,9,26,27). Investigation of these molecules has enriched our knowledge of the molecular genetics of carcinogenesis (26-28). For example, the product of *HMGA2* at 12q14.3 (previously *HMGI-C*) is a transcription factor named high-mobility AT-hook 2 (HMGA2) with physiological roles in embryological growth (29). However, upstream chromosomal disturbances, such as at 12q13-15, can influence the open reading frames of *HMGA2* may result in its overexpression and so neoplastic transformation. The miRNA let-7 can destabilise the *HMGA2* message, and so interrupt this transformation, therefore acting as a classic tumour suppressor. (30). Low expression of let-7 can be found in many cases of lung cancer (27), a presumed mechanism being that loss of the miRNA permits the oncogenic mRNA to develop the malignancy.

One of the key features of the development of a metastatic potential is the epithelial-mesenchymal transition (EMT)(31). miRNA-10 regulates this process in hepatoma cells, and so may be important in hepatocellular carcinoma, whilst several, such as miR-19 and miR-150, are linked with the transition in lung cancer (32,33). miR-181a is up-regulated, and miR-630 down regulated in hepatocellular carcinoma, and influence the EMT, possibly by an effect of transforming growth factor-β (34). An important aspect of the 'escape' of a tumour cell from the extracellular matrix and from other cells is loss of adhesive molecules such as integrins – the process of anoikis (35). Over-expression of miR-124 promotes anoikis of colorectal cancer cells *in vitro* and *in vivo* (36). A third aspect of tumour development and metastasis is angiogenesis,

the latter in promoting blood vessel development via growth factors such as VEGF and PDGF. Many miRNAs have been linked to this crucial process: for example, miR-9 and miR-494 have been described as pro-angiogenic, whilst miR-128 and miR-200 inhibit angiogenesis (37). Table 1 shows a small and selected collection of miRNAs and the cancers with which they are associated, table 2 shows some that are linked to the promotion or suppression of malignancy (38,39).

miRNAs in cardiovascular disease

Emphasis on miRNAs in this disease is often directed at the risk factors for atherosclerosis. One of the leading examples of a single gene defect bringing an enhanced risk of cardiovascular disease is that of familial hypercholesterolaemia. miR-34a may have role in the translation of $apoB_{100}$, miRs 185, 148a and 128-1 in that of the LDL receptor, and miR-22 in PCSK9 mRNA translation (40). Vascular smooth muscle cells (VSMCs) are induced to vasodilate by nitric oxide (NO), low levels of which may result from endothelial cell damage and so cause hypertension. miR-155 is reported to suppress endothelial NO synthase, whilst a network of miRNAs are involved in VSMC contractile/synthetic phenotypes (41). Numerous miRNAs are implicated in various aspects of the pathogenesis of diabetes, such as adipose differentiation, insulin resistance and secretion, and in β-cell development, whilst in particular, miR-25 is downregulated and miRNA 9 upregulated in diabetic neuropathy (42).

Given the involvement in miRNAs in the risk factors for atherosclerosis, it is therefore not unexpected that there should be roles for these molecules in the disease itself in general, and coronary artery disease in specific. The inflammation theory of atherosclerosis includes

activation of the transcription factor NF-κB, which plays an important part of factors that regulate inflammatory cytokines, matrix metalloproteinases and pro-coagulant tissue factor in atherosclerotic plaques (43). NF-κB may be activated by second messengers such as MAPK and Akt, which in turn can be activated by several miRNAs, including miRNA-195, miRNA-155-5p, miRNA-30e-3p and MiRNA-455-3p, but NF-κB may be inhibited by miR-127 (44). Numerous miRNAs are upregulated or downregulated in myocardial infarction (miR-195, miR-294), chronic heart failure (miR-133), hypertrophic cardiomyopathy (miR-204, miR-139-5p), dilated cardiomyopathy (miR-148a, miR-308b) and stroke (miR-574-3p)(45-47)

miRNAs as diagnostic tools

Improving our knowledge of the cell biology or pathophysiology of disease X or syndrome Y is all very well, but this knowledge must be applied to the care of our patients. There are numerous examples of differences in miRNAs in the malignant tissues compared with healthy tissues: miR-21 is higher expressed in malignant osteosarcoma tissue compared to nearby normal bone tissue (48), miR-199a-3p is down regulated, whilst miR-146b is highly expressed in thyroid cancer compared to normal thyroid tissue (49,50), miR-600 is down-regulated in breast tumours (51), let-7 and miR-935 are down-regulated in lung cancer tissues (52,53), and the ratio between miR-196a and miR-217 can help diagnose a malignant versus a benign pancreatic tissues (54).

Notwithstanding the value of biopsy or resected material, a blood test is far preferable, of which there are hundreds of examples, any of which also tell of the extent of the particular disease. Serum levels of miR-124 are reduced in pancreatic ductal adenocarcinoma compared to chronic pancreatitis and normal controls. Furthermore, levels in those with lymph node

metastases were a third those without these metastases and were less than half in those with advanced stages compared to early stages of the disease. However, this is not disease specific, as variants of miR-124 in DNA from peripheral blood may also be linked to gastric cancer (55,56). Combinations of miRNAs may also be useful: serum miR-152, miR-24 are lower whilst miR-222 and miR-150 are higher in uterine sarcoma compared to controls, and levels of all four miRNAs reflect the stage of the cancer (57). Breast and lung cancers are further examples of disease where good markers are needed. Serum miR-520f is lower in lung cancer, whilst variants of miR-27a and miR-146a, but not miR-196a2, in whole blood genomic DNA predict the presence of breast cancer (58,59).

miRNAs are also of interest in cardiovascular disease. Many miRNAs are altered in infarcted myocardial tissues, including miR-302b, whose target genes are the receptor for transforming growth factor β, and neurofibroin-1 (60). Serum miRNA-302b are higher in those having recently suffered an acute myocardial infarction, but, of course, so is troponin I, CK and CKMB (61). However, the miRNA provided the best area under the curve (AUC) of a receiver operator characteristic (ROC) analysis at 0.95 compared to 0.91, 0.72 and 0.82 respectively. It is unclear whether the increases in miR-302b are the consequence of the infarction or preceded the event. With strong correlations between the miRNA and the three cardiac markers (r= 0.79 – 0.92) it is tempting to speculate that levels arise from damaged cardiomyocytes. Early prediction of rejection following heart transplantation, defined by biopsy, is crucial, and may develop in up to 305 of recipients in their first year. In an initial cohort of 506, levels of miR-29 were raised compared to controls, fell in the years after transplant, but increased in those at risk of rejection (62). Analysing DNA from whole blood, levels of miR-126 are lower in diabetes, and lower still in diabetes plus coronary artery disease (CAD), whilst levels of miR-210 were the reverse: raised

five-fold in diabetes, but 20-fold in diabetes plus CAD (63). In the diabetics and the diabetics with CAD, miR-126 correlated inversely with fasting glucose and HbA1c, whilst in both groups miR-210 correlated with lipid indices, but in diabetes plus CAD, miR-120 also correlated with indices of hyperglycaemia.

The third great pathological process is autoimmunity, with rheumatoid arthritis (RA) to the fore, and again, with numerous examples of the presence of miRNAs (64,65). Increased levels of miR-146a in RA correlate with the ESR but not with the disease activity score, whilst miR-155 may have multiple role in disease progression (involvement with cytokines, apoptosis etc.) (66,67). But in the laboratory, we are not exactly short of cell or serum markers of this disease. It is therefore interesting to note that serum miR-201 in low in this disease, whereas serum miR-155 is high (67). Perhaps unsurprisingly, levels of both species correlate with swollen joint score, tender joint score, anti-CCP, RF, TNF-α, IL-1β, the ESR and rheumatoid factor, but not age, and both correlate strongly with disease activity. But the big finding is the both miRNAs out-perform all the other laboratory indices described above in a multivariate analysis to distinguish RA from controls. Could these miRNAs become routine markers for this common disease? There is also literature on other autoimmune diseases such as SLE, Sjogren's syndrome and inflammatory bowel disease (68-71).

miRNAs in management

Having made the diagnosis, can miRNAs help with managing the disease? There are ample examples of miRNAs being linked to disease severity (table 3)(73-77). Jimenez-Lucena and colleagues measured levels for four miRNAs in 462 patients with cardiovascular disease but

no diabetes (78). After a median follow-up of five years, 107 developed diabetes and 30 developed pre-diabetes. High levels of miR-150 and miR-30a-5p but low levels of miR-15a and miR-375 were linked to the development of diabetes, findings that could be used to evaluate the risk of developing the disease, which may improve prediction and prevention among individuals at high risk for T2DM. But as with rheumatoid arthritis, the laboratory offer many potential disease markers in diabetes. Our colleagues from Spain performed a multivariate analysis, finding that all the standard clinical and demographic data together gave a ROC AUC of 0.71, rising to 0.76 when OGTT-derived indices were added, but the most significant was when clinical variables were added to the miRNAs, with an AUC of 0.79. Use of these markers may therefore help clinical decision-making in determining which patients are at greatest risk of disease progression, and so warrant the most urgent attention. The ultimate disease progression is non-survival, and miRNAs can also help determine those cancer patients with the worse outcome survival (table 4).

So, we know that miRNAs can help with a diagnosis, and give an indication of disease progression, but what of treatment? Numerous cell biology studies have shown that miRNAs can act as both tumour suppressors and oncogenes, and so make good potential targets, as in the case of miR-486 and myeloid leukaemia (70). miR-370 can interact with the epidermal growth factor receptor (EGFR), miR-140 with the fibroblast growth factor receptor, whilst miR-101, miR-200c and miR-338 may all interact with nuclear transcription factors and so influence EMT, invasion and metastasis (79). It follows that all these miRNAs give direction regarding potential manipulations, and so treatment (80).

An example of this is the case of the oncoRNA miR-21. Classic cell biology and animal experiments showed that knockdown disrupts glioma cell growth (81), possibly by rescuing

tumour suppressors and/or involvement in other pathways such as that of the EGFR. Zhang et al showed that the combination of a monoclonal antibody to the EGFR (nimotuzumab) and an inhibitor of miR-21 is superior to single agent therapy (82). Whilst synthetic anti-sense nucleic acids are potential blocking agents, others have pseudo enzymatic catalytic activity (i.e. ribozymes) to degrade their target (83). Animal models work: inhibition of miR-208a in a rat model improves cardiac function and survival during heart failure (84), inhibition of miR-10b in a mouse model inhibits mammary tumour metastases (85), and inhibition of miR-21 in a mouse model of SLE reduces splenomegaly (86). The next step is to devise a safe and effective delivery system (such as nanoparticles and recombinant viral systems) so that these agents can enter the clinic (87,88). This has been achieved in a primate model, where a locked nucleic oligonucleotide complementary to miR-122 leads to suppression of a hepatitis C viraemia (89), and small phase 1 and 2 clinical trials of anti-miRNA oligonucleotide in our own species show very promising results (90,91). Other promising data comes from a variety of sources. Mipomersen is a 20-nucleotide antisense oligonucleotide engineered to inhibit the mRNA for $apoB_{100}$: subcutaneous injections resulted in ~3% reductions in LDL and $apoB_{100}$, and ~27% reductions in Lp(a) (92). It could be argued that small interfering RNAs (siRNAs) are different to miRNAs, but nevertheless they too can have direct clinical effect, as in reducing levels of LDL cholesterol (93,94), in stabilising advanced solid tumours (95), and in reducing the effect of an induced respiratory syncytial virus infection (96). We await other trails, and so marketing authorisations (97).

Long non-coding RNAs

This group comprises long (greater than 200 nucleotides) non-coding RNA (lncRNAs) molecules. Considerably less is known about lncRNAs, but, as there are estimated to be 50,000 – 60,000 such molecules, they may, collectively, be as important as miRNAs (2,3,98,99). Despite the much larger number of lncRNA species in the genome that by far exceeds that of miRNAs and protein coding genes, there is as yet no clear numbering system comparable to that of the miRNAs, and so are named by abbreviation, often reflecting their origin. Of the thousands of lncRNAs that have been identified, and the purpose of the vast majority remains obscure, although many are found in certain cancers and so may also (like miRNAs) have a role in carcinogenesis. Nevertheless at least six different functions for lncRNAs have been postulated: binding to intron/exon pre-mRNA boundaries in the nucleus, recruitment of mRNA editing enzymes (also within the nucleus), inhibiting mRNA translation at the ribosome, interference with the interaction between mRNA as a transport protein Staufen, acting as a sponge to mop up miRNAs, and masking mRNA sequences that enable its binding to the RNA-induced silencing complex. A further potential function is the generation of micropeptides resulting from transcribable open reading frames, and interaction with histones and chromatin-associated proteins (2,98).

An example of the potential multiple roles of lncRNAs is that of urothelial carcinoma-associated 1 (UCA1). Its oncogenic functions have been studies in colorectal, breast and bladder cancer, and acute myeloid leukaemia (100). It increases signal transduction pathways in prostate and hepatocellular cancers, promotes tumour progression by targeting miR-193a-3p and miR-204-5p in lung and colorectal cancer respectively, and have role in regulating haem biosynthesis and erythropoiesis (101). Other examples include a functional variant in the lncRNA GAS5 which is linked to gastric cancer (102) and that of lncRNA Xist, which predicts

the presence of lymph node metastases in oesophageal cancer (103), whilst several lncRNAs are implicated in the metastatic development of breast cancer (104). The wonderfully-named HOTAIR is linked to breast, oesophageal, gastric, colorectal, lung and renal cell cancers, whilst MALAT1 (metastasis associated lung adenocarcinoma transcript) has effects in lung and bladder cancer, osteosarcoma and lymphoma (98). Around the other way, lncRNAs H19, MALATI, SNHG16, TIG1, UCA1, TINCR and Linc-UBC1 all have links to bladder cancer (99).

As regards atherosclerosis, lncRNAs have been linked to autophagy in endothelial cells and vascular smooth muscle cells (TGFB2-OT1), endothelial differentiation (SENCR), inhibition of the expression of ApoA1 and the formation of HDL (APOA1-AS), and the regulation of cholesterol homeostasis (RP5-833A20.1)(8,46). Levels of circulating lncRNAs CoroMarker, BATs and IL21R-AS1 can differentiate patients with coronary artery disease (105), whilst lncRNA LIPCAR marks cardiac remodelling and predicts mortality in patients with heart failure (106). Unsurprisingly, numerous commentators discuss the likelihood that, like miRNA, various lncRNAs could be therapeutic targets, citing numerous examples of lncRNA involvement in the EMT and in atherogenesis (107,108). The lncRNA CHROME is an example that may come to market, with a development that parallels that of mipomerersen and inclisiran in hypercholesterolaemia (92,94) with basic cell biology and animal models (109).

NcRNAs and the laboratory

The technology to analyse miRNAs and lncRNAs is established and should transfer to a routine NHS or private molecular genetics laboratory without too much difficulty. miRNA can be measure in paraffin blocks, fresh tissues, cell extracts, serum, plasma, semen, faeces, lens

tissue, saliva and vitreous humour, as can lncRNA in most (110-115). It may be only a matter of time before new standard operative procedures will be written.

Conclusions

These two major forms of ncRNA are clearly important new markers of various disease and their development, and many provide therapeutic opportunities. Other forms, such as circular RNAs, may also be important (2,4,5,116,117). However, their biology is very complicated are far from well understood. As indicated in the text, there are numerous examples of single diseases where links with multiple ncRNAs and been described, and *vica-versa*, there are several instances where a single ncRNA influences the pathophysiology of several diseases (38-40,44-46,77,108,118). It is also clear that these miRNAs and lncRNAs influence each other (109). Nevertheless, the likelihood that one or more of these molecules will enter routine clinical service cannot be ignored.

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Table 1: miRNA changes in selected cancers

Cancer	miRNA down-regulated / under-expressed	miRNA up-regulated / over-expressed
Lung	Let-7, 9, 25, 29, 32, 124, 142, 143, 145, 155, 181, 183, 218	17, 21, 26a, 92, 155, 205
Breast	Let-7, 125a, 126a, 143, 145, 155, 181, 200, 218, 342	9, 10b, 21, 26a, 125, 146, 155, 372, 373, 520
Prostate	Let-7, 15a, 16-1, 101, 125a, 125b, 145, 146b, 181, 205, 218	21, 106b-93-25 cluster, 146
Colon	Let-7, 29, 145, 155, 342	21, 26a, 34b/34c, 106b-93-25 cluster, 155

n.b. This table is intended to be neither comprehensive not exhaustive

Table 2: miRNAs and cancer progression

Malignancy promoted

miR species up-regulated	Cancer	Target
miR-155	Breast	Von Hippel-Lindau (a tuour suppressor)
miR-494	Lung	Phosphatase and tensin homolog (PTEN, a tumour suppressor)
miR-221/222	Thyroid	Adiponectin receptor 1
miR-774-3p	Laryngeal	Phosphatase and tensin homolog (PTEN, a tumour suppressor)

Malignancy suppressed

miR species down-regulated	Cancer	Target
miR-101	Oral (tongue)	A component of polycomb repressive complex 2 (has histone methyltransferase activity)
miR-876-5p	Head and neck	Vimentin (cytoskeleton component)
miR-199a-3p	Liver	VEGF and its receptor
miR-454	Pancreatic	LRP6 (low density lipoprotein receptor-related protein 6)

From references 38,39 and others.

Table 3: miRNAs and disease severity

Disease	miRNA	Link	Reference
Rheumatoid arthritis	155/201	Strong links with disease activity score	67
Breast cancer	155	Levels increase with stage	72
Oral squamous cell carcinoma	125b	Low levels linked to increased stage	73
Lung cancer	198-5p	Low levels linked to increased stage and to vascular invasion	74
Prostate cancer	424-3p	Low expression linked to aggressive phenotype	76
Lung	miR-141	High levels linked to larger tumour size, lymph node metastases and advanced stage	77
Diabetes	150/30a-5p/ 15a/375	Development of pre-diabetes or diabetes	78

Table 4: miRNAs and survival outcome

Disease	miRNA	Link	Reference
Chronic lymphocytic leukaemia	650	High levels linked to longer survival and longer time to treatment	24
Osteosarcoma	21	Low levels inked to por disease-free survival and overall survival	48
Oral squamous cell carcinoma	125b	Low levels linked to poor survival	73
Lung cancer	198-5p	Low levels linked to poor survival	74
Colorectal	20a	High levels linked to poor survival	75