How miR34-a could be used as a therapeutic against cancer

Content

1 Abstract

Cancer is still a huge problem in the world and although many different medicine exists the perfect treatment remains to be found. Nowadays, microRNAs (miRNAs) are thought to be used as a new kind of medicine to counteract cancer. MiRNAs have shown to play an important role in various cell processes by binding to their respective complementary mRNA sequence and in this way block the translation of the mRNAs into proteins. In many diseases, including cancer, it was found that the miRNAs were dysregulated. In cancer one particularly miRNA was often found to be downregulated, this is miRNA-34a (miR-34a). The mRNA targets of miR-34a are often involved in the stimulation of cell proliferation and if these are not properly regulated, it will result in uncontrolled cell growth. Since miR-34a is involved in the downregulation of these proteins the thought occurred that miR-34a mimics could possibly work as a therapeutic. This drug was already going through medical tests phase I, but was terminated due to the fact that some patients had a severe immune reaction during the protocol. However, more miRNAs are going through test phases and with the use of the right transport systems they could possibly evolve to a good medicine against cancer. Maybe have the power to eradicate cancer from the world completely.

2 Introduction

More than 9,6 million people died because of cancer in 2018 and it is estimated to become the leading cause of death in the 21st century[1]. There are different types of cancer, but all show certain similar characteristics. Cancer is described as the uncontrolled growth of cells due to one or more mutations in genes mostly transcribing for proteins involved in cell regulation[2]. Nowadays, several treatment strategies against cancer are available. The most common ones are surgery, chemotherapy, radiotherapy and since recently immunotherapy, which shows great promises. However, the great amount of deaths among cancer patients show that these therapies are still not 100% effective and each treatment has its own unwanted side effects. This means that there is still a search for new, more effective medicines to cure cancer with fewer or no side effects. MicroRNAs (miRNAs) could be exactly what we are looking for. Their potential as cancer therapeutics has gathered a lot of interest and these therapeutics are already used in patients suffering from cancer.

miRNAs are non-coding RNAs of ~22 nucleotides. It was only since 1993 that the first miRNA, called *lin-4*, was identified in *Caenorhabditis elegans*[3]. Since then the field of miRNAs has greatly expanded and many new miRNAs have been discovered[4]. Their sequences are highly conserved across species, which already suggests that they might have important regulatory functions. This suggestion has been validated in several studies in which it became clear that certain miRNAs are involved in main cellular processes such as proliferation, apoptosis and differentiation[5].

miRNAs bind to their target mRNAs to direct the posttranscriptional repression of these mRNAs[6]. One specific miRNA generally has several mRNA targets, making that the expression of one specific miRNA could result in different effects in the cell. If miRNA binds to mRNA, the mRNA will not be translated to a protein and could possibly be degraded.

Research found that the miRNA levels were dysregulated in several diseases. For example, miR-103 was found to be upregulated in obese mice and downregulation of miR-143 and -145 was described in heart diseases[7,8]. Also in cancer, many examples were found in dysregulated miRNA levels[9]. This suggests that miRNAs play a role in the development of cancer, and that restoring the normal miRNA concentrations could help in treating cancer.

In summary, miRNAs (I) play a large role in different pathways (II) are thought to be involved in the development of cancer and (III) have an effect on several target mRNAs. All of the above led to the fact that miRNAs could be a good candidate to use in the development of a new type of drug. The drug could either be a (I) miRNA mimic, to restore the function of their original miRNA or an (II) anti-miRNA, to make their complementary miRNA target non-functional[10].

One of the most studied miRNAs involved in the development of cancer is miR-34, of which 3 different types are specified by the human genome, namely miR34a, miR34b and miR34c. MiR-34 is shown to be dysregulated in different cancer types and is already made into a drug against cancer, which has been tested in cancer patients[11]. Since miR-34a shows better tumour suppressor characteristics than its sisters[12], this report will focus on miR-34a. In this review I will show how miRNA-34a makes a good candidate to use as a therapeutic against cancer.

3 miR-34a, "the Guardians little helper"

Research has shown that especially miR-34a is connected to the tumour suppressor gene, *TP53* and encodes for the protein p53. This protein is also called the "Guardian of the genome", since it is involved in many different processes in the cell that counteract tumour development[13]. This is why miR-34a has been labelled "the Guardians little helper" and would be an interesting candidate to be investigated with respect to how it might be involved in tumour suppression mechanisms.

In this chapter the function of miR-34a will be described in great depth. The biosynthesis, sequence, its relationship to p53, targets and involvement in cancer development will be described.

3.1 Function of miR-34a

The general function of miR-34a is to stop translation of its mRNA targets. The miRNAs recognize their targets with the so called "miRNA seed" which are the bases 2-8 in the mature miRNA. These are complementary to the binding site on the target mRNAs[14]. The mRNAs have a 3' untranslated region (3'UTR) and in this 3'UTR lays the binding site for the microRNAs[15]. There are two possible outcomes after the binding between mRNA and microRNA, either there is a translational repression or degradation of the mRNA (*Fig. 2)*[16]. Both resulting in the downregulation of the protein. It is predicted that over 30% of all protein coding genes are controlled by miRNAs[17], which also supports the fact that miRNAs play an important role in the cell.

3.1.1 Sequence and location in the human genome

In several types of human cancers a deletion was found of chromosome *1p36*[18]. Therefore, it was assumed that tumour suppressor genes might be encoded in this area of the genome and a search for such genes commenced[22]. Eventually a gene was identified, encoding for miR-34a. This discovery strongly suggested for the first time that miR-34a could be a potential tumour suppressor gene. With the use of bioinformatics and techniques like ChIP-PET, a p53 binding site was found in the CpG island (*Fig. 1A)*. The seed of the miRNAs are always located at 2-8 nucleotides of the mature miRNA, so this seed sequence was easily found (*Fig. 1B*). This miRNA seed would give an extra identification about what the targets are for this miR34-a.

3.2 Biosynthesis of miR-34a

The biosynthesis of miRNAs follows a specific pathway (*Fig. 2B*). The miRNA genes are first transcribed as primary miRNA (pri-miRNA) by RNA polymerase II, wherein the ~30 kbp intron will be spliced out. The pri-miRNA forms a long hairpin structure (*Fig. 2A*)[19]. Drosha, a nuclear RNase III enzyme will subsequently cut the pri-miRNA and a shorter hairpin with a two-nucleotide 3' overhang will form, called precursor miRNA (pre-miRNA)[19]. The pre-miRNA is translocated to the cytoplasm by Exportin 5 (XPO5), a protein that recognizes the 3' overhang of the pre-miRNA[20]. The terminal loop is cut off by the RNase II Dicer, leaving a small RNA duplex[21]. This duplex is then loaded onto RNA-induced silencing complex (RISC). This protein complex stabilizes the miRNAs and helps them find their targets[22].

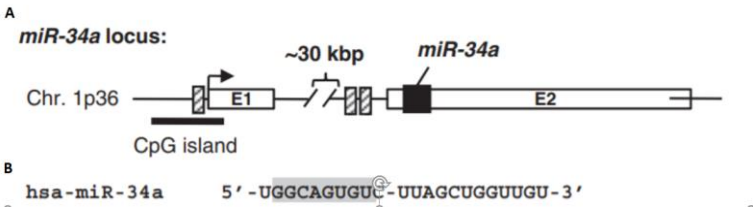

Figure 1: A. Sequence and location in the human genome of miR-34a gene. The dashed boxes are p53 binding sites, the arrow indicates transcription start site (TSS), white boxes are exons and the black box is the mature miR-34a. The primary miRNA is produced by splicing the ~30kbp part in between E1 and E2 out[24] *(further processing described in 3.2). B. Mature miR-34a sequence, grey part shows seed sequence* [26].

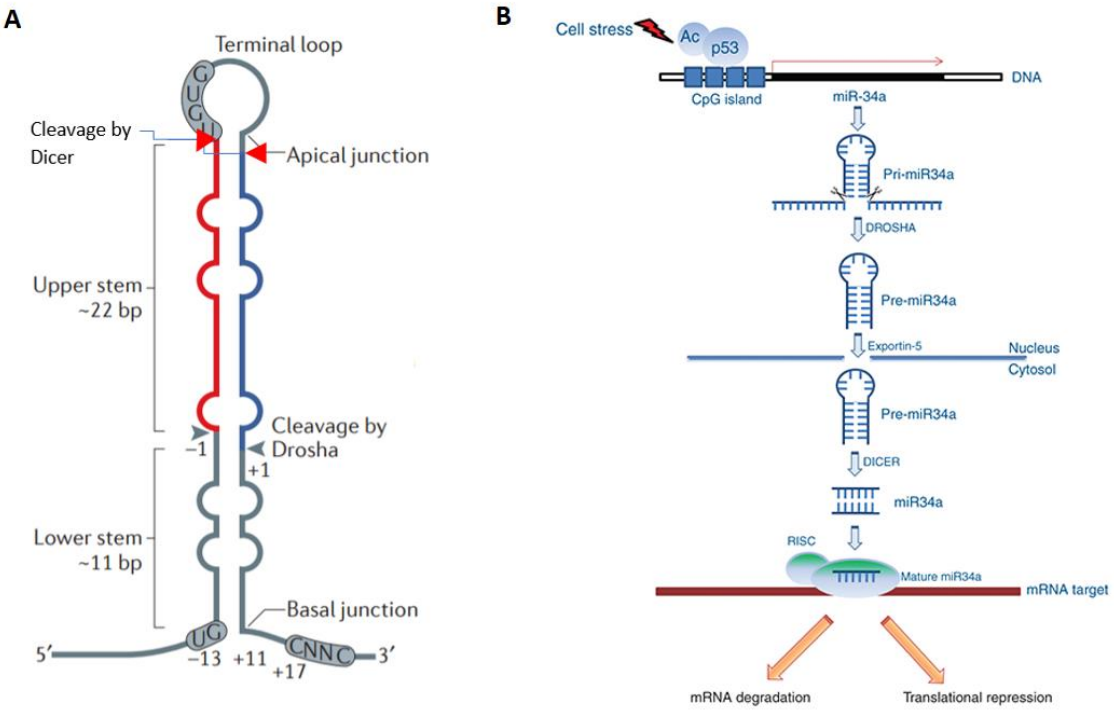

Figure 2: A. Pri-miRNA with a stem of 33-35 bp, a termial loop and single stranded RNA segments at 5' and 3' end. Cleavage sites for both Drosha and dicer are indicated with grey and red arrows respectively[79]*. B. A global overview of the biogenesis pathway of miR-34a*[11].

3.3 Upregulation of miR-34a by p53

Stress signals like DNA damage results in activated p53, the protein p53 transforms into an activated state with the help of the acetyltransferase p300, which acetylates p53 using its acetyltransferase activity. Upon acetylation of p53 on its C-terminus, the sequence-specific DNA-binding activity of p53 is activated, allowing this regulatory protein to bind to specific DNA regions. When p53 is bound to DNA it results in the transcription of the corresponding gene. How transcription is enabled exactly is not yet known[23] . The target genes of p53 encode for proteins that function in regulating cell-cycle progression, DNA repair and/or apoptosis[13].

It was shown that the promoter of the miR-34a gene contains a p53-binding site and is a direct target of p53. After DNA damage the gene is upregulated[24,25]. There are a few advantages to transcribe miRNA. One is that miRNA will also work on the mRNA levels that are already transcribed, as a post transcriptional regulator. Furthermore, miRNA does not require additional translation, which can save a lot more time and energy[26].

There is a lot of evidence that miR-34a is upregulated by p53, but independent pathways in which miR34a gets upregulated were also found[27].

3.4 Targets of miRNA-34a

miR-34a have many targets; the outcome of bioinformatic analysis suggested that miR-34a has more than 100 mRNA targets. However the experimentally validated targets of miR-34a are shown in *Figure 3A*[11,28,29]. Many of these targets are part of the p53 network genes[30]. Due to time restrictions we will only focus on a few to show the importance of miR-34a and how it is involved in cellular pathways that are related to tumour suppression.

3.4.1 SIRT 1

Silent information regulator (SIRT) 1 is a deacetylating protein. This deacetylase is involved in the regulation of apoptosis and evidence suggests that its gene could be an oncogene[31]. SIRT 1 regulates apoptosis by deacetylating p53 at its C-terminal, resulting in the inactivation of p53[30]. The upregulation of miR-34a results in a lower concentration of SIRT 1, indicating that the two are connected. miR-34a binds to the mRNA of SIRT 1, resulting in a reduced translation of the SIRT1 mRNA. Lower protein expression of SIRT 1 makes that p53 deacetylation is prevented, which leaves p53 in its active form and fulfils its normal role[30]. This is also why the transcription of miR-34a by p53 represents a positive feedback loop, since miR-34a inhibits an inhibitor of p53 (*Fig. 3B*)[30].

3.4.2 BCL-2

A statistical analysis revealed an inverse correlation between miR-34a and BCL-2 in which the downregulation of miR-34a coincided with an upregulation of the BCL-2 gene (*Fig. 3B)*[32]. Furthermore, target scans showed that BCL-2s mRNA is one of the many targets of miR-34a; this was supported by data showing a decrease in BCL-2 levels in U87 cell lines that were transfected with a miR-34a mimics[33]. BCL-2 is a negative activator for the pro-apoptotic proteins BAX and BAK and will block their functional activity. BCL-2 does this by binding to BH3, making BH3 unable to activate BAX and BAK proteins[34]. BAX and BAK act on the mitochondrial membrane and make it permeable, resulting in the release of cytochrome C, which is an important signal in the apoptosis process[35]. Increased BCL-2 protein levels are often observed in cancer cells, this leads to cells not easily going in apoptosis and to their continuous uncontrolled growth[36].

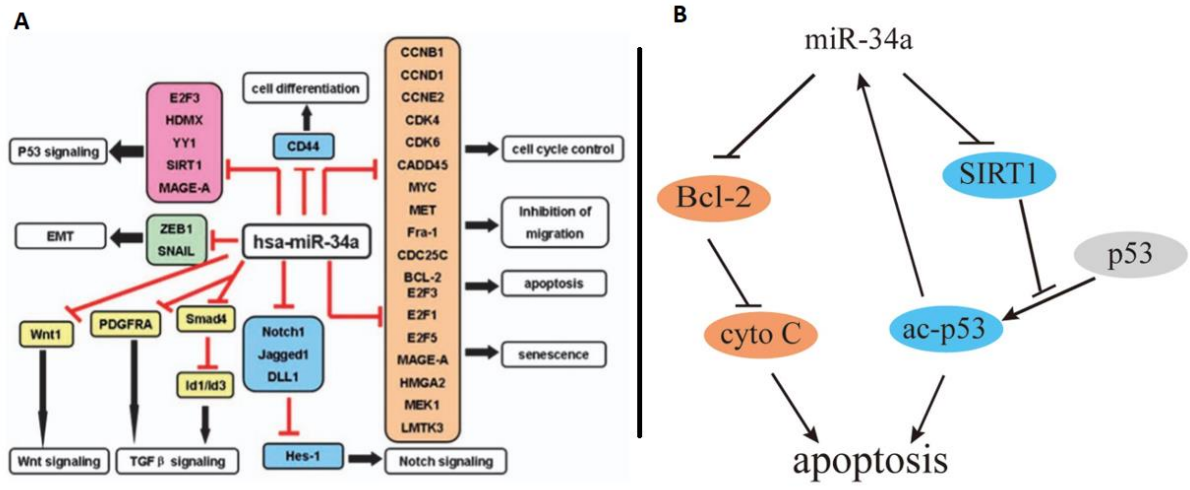

Figure 3: A. A global overview of the targets of miR-34a. The different colours indicate the different groups of proteins that have a specific outcome when downregulated by miR-34a, the cellular reactions are indicated in white boxes[29]*. B. Specific pathway to show the relation between BCL-2, SIRT 1 and p53. The different colours clarify the two different pathways*[80,49]

3.4.3 E2F3

E2F3 is a transcription factor and a member of the E2F family. This transcription factor is necessary for the induction of the S phase in the cell cycle. E2F3 helps transcribe genes that enables the proliferation of cells[10]. Overexpression of E2F3 is observed in different types of cancer and therefore the gene encoding E2F3 is considered as an oncogene[37]. Overexpression of miR-34a resulted in less E2F3 protein, which suggest that the E2F3 transcript a complementary binding site to the miR-34a seed[38].

3.4.4 MDM4

The MDM4 is a negative regulator of p53, it binds in the transactivation domain of p53 and this way inhibits the transcriptional activity of p53, making it unable to regulate gene expression[39]. Binding of MDM4 to p53 eventually result in ubiquitination and consequent degradation of ubiquitinated p53. It has been shown that transgenic mice in which the expression level of MDM4 was increased developed tumours[40]. This suggests that MDM4 is involved in the development of tumours. Unlike miR-34as other targets, it does not bind to the 3' UTR of the mRNA of MDM4. Instead a miR-34a binding site was predicted to be in MDM4s open reading frame, the sequence of this binding site conserved in MDM4 from other species. Experimentally, this prediction was confirmed. Showing that *MDM4s* mRNA is a direct target of miR-34a. The same study found that overexpression of miR-34a led to inhibition of expression of the MDM4[41].

3.4.5 YY1

Yin Yang 1 (YY1) is a transcription factor that can either act as a repressor or an activator of gene transcription. This protein is also known to be involved in cancer development and is overexpressed in human colon cancer cells[42]. However, because of its dual role, the function of YY1 is not as clear as the other proteins described above. For instance, in some cancer types YY1 levels were strongly elevated while in others they were significantly down[43]. One of the functions of YY1 is to interact with p53. This interaction prevents p53 from binding to its coactivator p300, resulting in a non-activated p53[44]. Also, *YY1* transcript showed a conserved 3' UTR binding site for miRNA-34a and in *in vitro* experiments the downregulation of miRNA-34a resulted in upregulation of YY1[45]. These results indicate that *YY1*s mRNA is a direct target for miRNA-34a. Binding of miRNA-34a to the 3' UTR binding site in the transcript of *YY1* results in less production of YY1. This leads to p53 not being bound to YY1, that can then bind to its coactivator p300.

3.5 How miR-34a is involved in cancer

From the previous paragraph it can be concluded that miR-34a has many targets that are involved in cell proliferation processes. Also, many of these targets are upregulated in cancer cells. Further investigation showed that after DNA damage several miRNAs were upregulated of which miR-34a was most upregulated[25]. This is another indicator that it is an important miRNA in the regulation in tumorigenesis.

3.5.1 The effects at the cellular level of upregulation of miR-34a

Cells overexpressing miR-34a show a senescence-like morphology[27]. Senescence is a stress response that results in a permanent proliferative arrest of the cell. Upregulation of miR-34a also induces apoptosis. This was shown by transfecting cells with a small interfering RNA (siRNA) that corresponds to the sequence of miR-34a, so this siRNA has the same function as miR-34a. An increase in cells with sub-G_1 DNA content was observed after this treatment, indicating that the cells were in an apoptotic state[46]. This information shows that a high level of miR-34a supports apoptotic and senescence states of the cell and blocks processes that are linked to cell differentiation and proliferation, two processes that are strongly linked to tumorigenesis[2].

3.5.2 Downregulation of miR-34a in cancer cells

Since miR-34a is associated with tumour suppressive mechanisms such as apoptotic and senescence processes, its gene is thought to be an “anti-oncogene”. Downregulation of miR-34a is often observed in cancer cells. One study found that 11 out of 15 pancreatic cancer cell lines showed a 10-fold reduction of miR-34a[25]. Downregulation of miR-34a could be cause by CpG methylation of the promotor of the miR-34a gene. Such methylation was observed in breast, lung, colon, kidney, bladder and pancreatic cancer cell lines[47]. It would be logical that downregulation of miR-34a is a consequence of mutations in the p53 gene (*TP53*), which are also often seen in cancer cells. However, as downregulation of miR-34a was both found in cancer cells with and without a functional p53, miR34a levels are not necessarily affected by mutations in *TP53*[48].

4 MiRNA-34a as a therapeutic

In the previous chapter I discussed the upregulation of miR-34a by p53 (a known factor in cancer development), the important role that miR-34a plays in the cell (because of its many targets) and in cancer development.

Since miR-34a is downregulated in many cancer types and because it regulates the levels of many proteins that are linked to the development of cancer, it seems logical to assume that restoring the miR-34a concentration via miR-34a mimics could support normal cell functioning. Also there are advantages in using miRNAs as therapeutics since they work on mRNA levels and do not need to be translated to functional protein to be active: they can directly act on their target mRNAs. In principle, this is of course a much faster route.

4.1 Experiments showing effectiveness of miR-34a introduction

In mice that were derived from the human colon cancer cell line (HCT 116) and colon carcinoma cell line (RKO), suppression of tumour growth was observed after the introduction of miR-34a. miR-34a mimics were purchased from ambion and were introduced in the mouse by using atelocollagen as delivery system, which is a collagen without the telopeptide regions. When tumour was 50-100 mm^3 the surrounding tissue were injected with miR-34a/atelocollagen. Already 14 days after the treatment a large difference in tumour volume was detected and necrotic tissue was found, showing that miR-34a suppresses tumour growth (*Fig. 4*). Also the introduction of miR-34a in a p53-knockout cell line represses cell proliferation, which indicates that miR-34a also works independently of p53[49].

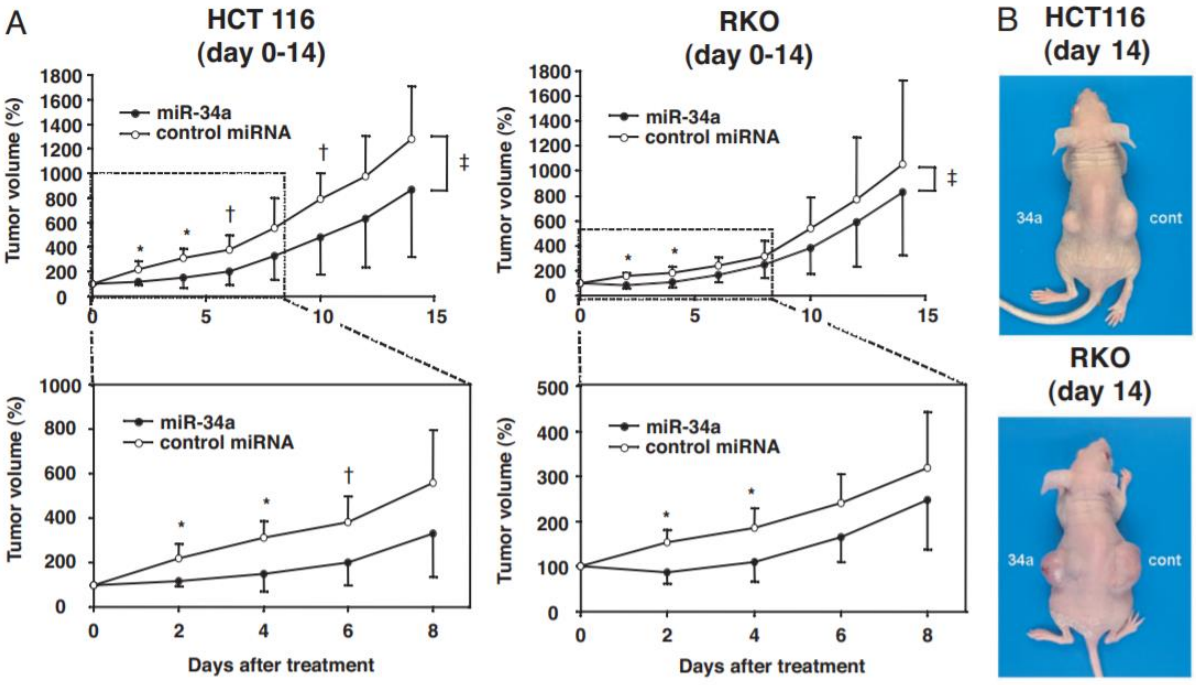

Figure 4: A. Tumour volumes of both HCT 116 and RKO mice were measured after the miR-34a/atelocollagen treatment. The 100% tumour volume is the volume of the mouse when the treatment started. A difference is shown in tumours that were treated with miR-34a or with control miRNA. B. A visual representation of the HCT 116 and RKO mice, 14 days after the treatments. The right was treated with control miRNA and the left with miR-34a[49].

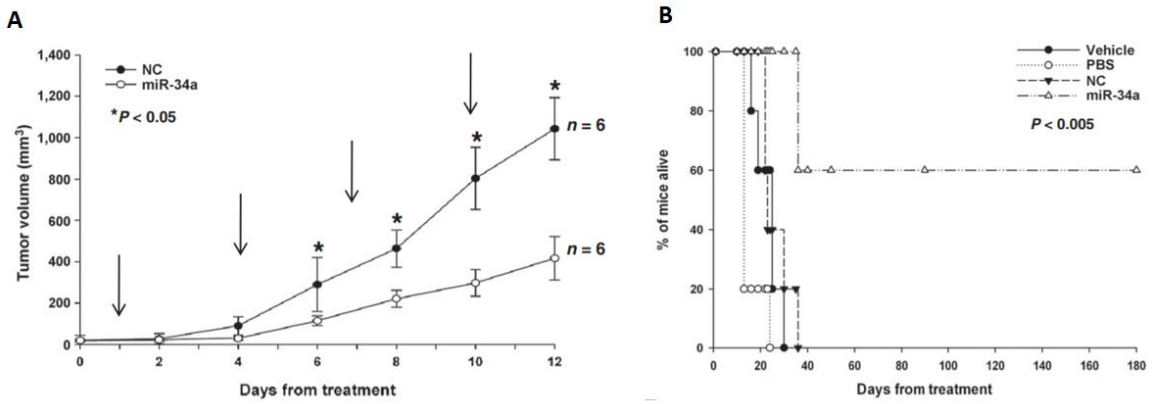

Figure 5: Results in SCID mice xenografts with MM cells. A. Tumour volume in mm^3 is set against days after the treatment. Arrows point to the days when the mice were injected. A comparison is made between mice treated with miR-34a and scrambled miRNA (NC). B. Percentage of mice alive are set against days after treatment. A comparison is made between mice that were treated with an empty vehicle, PBS buffer, NC or miR-34a as can be seen in the legend[52].

The same kind of experiment was done in severe combined immunodeficient (SCID) mice bearing human multiple myeloma (MM) cells. SCID mice are excellent to make xenografts with human cells and tissues, because they do not react to these different cells/tissues due to their immunodefiency[50]. In this experiment they used neutral lipid emulsion, which has previously shown to be effective in carrying miRNAs to tumour cells[51]. SCID mice were injected with NLE vesicles carrying miR34a mimics which were purchased from Ambion. Inhibition of the tumour growth was established after 4 injections with 3 days in between the injections (*Fig. 5A)*. Also an intense prolongation was observed in mice that were treated with miR-34a (*Fig. 5B)*[52].

4.2 Delivery systems of miR-34a

The results presented above were only two example of many more. They showed promise of employing miR-34a mimics as therapeutics. However, one of the main obstacles to overcome is the fact that miRNAs are not stable in the cell and will be degraded fairly fast, while their uptake is also poor. In other words, for miRNAs to be effective in *in vivo* treatment strategies, there is a great need for a properly working delivery system that is highly effective and low to non-toxic[53]. Two types of delivery systems will be discussed now, to show what the possibilities are in this area, but of course many more exist.

4.2.1 Adenoviral vectors

Adenoviral vectors are already being used in delivery systems for several drugs. An adenoviral vector consists of a construct carrying a gene of interest secluded in a capsid that will be injected in or around tumours. The adenoviral vector will be taken up in the host cell after which the DNA construct will find its way into the nucleus. Here the construct, including the gene of interest, will be transcribed by using its own promotor[54].If this is done with a miR-34a gene, it will be transcribed from its own promoter and the miR-34a transcript levels will be elevated in tumour cells in which these levels were initially down. In this way, the miR-34a levels become equal to those in “healthy” cells[55]. *Figure 6A* presents the adenoviral constructs used in this study. This experiment actually wanted to show that the combination of miR-34a and IL-24 is more effective in treating cancer cells, but in this experiment it also becomes clear that adenoviral vectors are effective in making the expression level of miR-34a higher in cancer cells (*Fig B.*)[56].

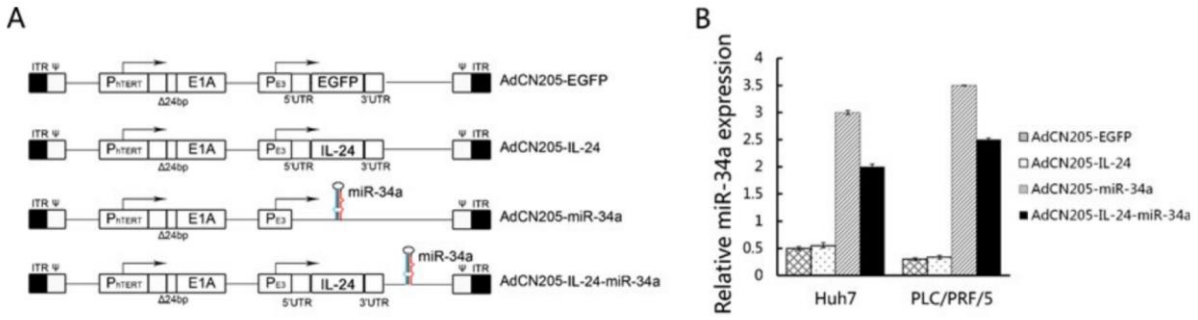

Figure 6: A. Adenoviral constructs used in experiment. From left to right it starts with the inverted terminal repeat (ITR), after that a phTERT promotor is found. hTERT protein is involved in cancer biogenesis and is mainly expressed in cancer cells. This makes that the promotor is ideal to use in cancer therapy[81]. Early region 1 A (E1A) is transcribed after phTERT activation and activates P_{E3} promotor making that the gene downstream for this promotor will only be transcribed in tumour cells[82]. B. The relative miR-34a expressions for two different HCC cell lines, Huh7 and PLC/PRF/5, are presented. The adenoviral vectors that include miR-34a showed the highest expression of miR-34a, which means the adenoviral vector worked in delivering[56].

Although these results are promising, there are a few downsides in using adenoviral vectors. It is known that they can induce a strong innate immune response[55]. Also, many patients have already been infected with an adenovirus during their life, which makes them immune to this type of delivery system[57]. Furthermore this approach is linked to toxicity, since it could result in a cytotoxic T cell immune response and would therefore be hard to use in therapies[58]. Some approaches are currently being explored to make this technique less toxic and more efficient, for example by removing some genes of the adenoviral DNA[59].

4.2.2 SNALP

Using Stable Nucleic Acid Lipid Particles (SNALPs) is one of the promising delivery systems (*Fig. 8*). DNA and RNA are negatively charged due to their phosphate groups, making them anionic. SNALPs are provided with cationic lipids to stabilize the anionic miRNA sequence and to improve the association between the miRNAs and SNALPs. The positive charge of the vesicles also enables cell uptake[60]. However, using only cationic lipids is unfavourable, since they are toxic[61]. This is why neutral lipids are also being used in their formation. Polyethylene glycol (PEG)-conjugated lipids are used for steric stabilization of the liposomes and will induce the formation of vesicles[62]. SNALPs are stable in serum, have a high vesicle loading capacity and have good transfection efficiency[63].

SNALPs could encapsulate 160 µg miR-34a per mg lipids. SNALPs containing miR-34a were injected 5 times 3 days in between each injection. Liver and kidney were harvested and investigated with respect to toxicity of the vesicles; the SNALPS had no toxic effects, as both organs showed normal histologic levels. Tumours were obtained in which 50 % apoptosis without evidence of necrose, meaning these drugs were highly efficient (*Fig. 6*)[64]. Furthermore, an increased level of miR-34a was found in treated tumours. This all together shows that SNALPs efficiently work as a delivery system.

Since SNALPs show high efficiency and low toxicity, they could definitely be a candidate to use as delivery system of miR-34a mimics in human cancer patients.

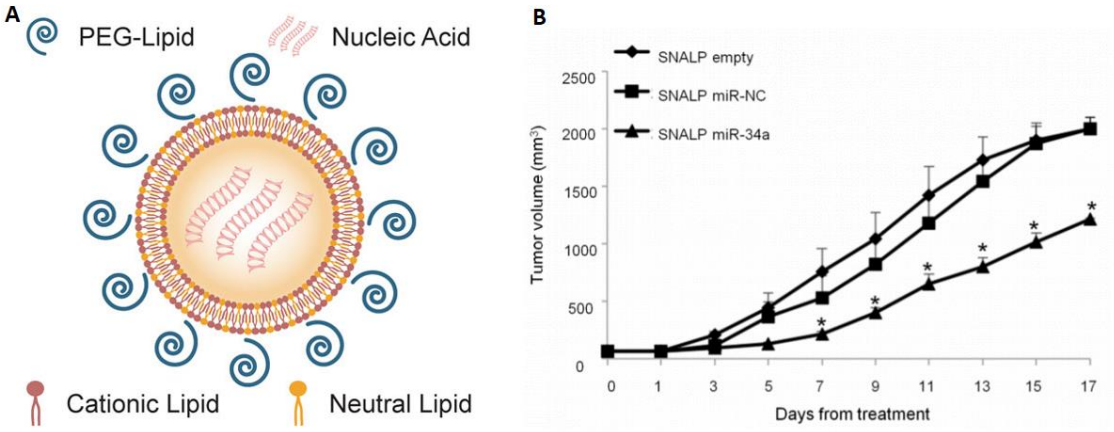

Figure 7: SNALP as delivery system. A. Schematic picture of how a SNALP looks like[84]. B. Experimental outcomes with the use of SNALP as delivery system. A comparison is made between an empty SNALP, a SNALP encapsulating miR-NC and SNALP miR-34a. Tumour volume in mm³ is set against days after treatment[64].

SMARTICLES

The systems used for miR-34a delivery as a therapeutic are called SMARTICLES. They are based on the principle described above. Two of the key features are their pH sensitivity and the use of both anionic and cationic lipids. The pH sensitivity is enabled by the use of two cholesterol derivatives, CHEMS (anionic) and MO-CHOL (cationic)[65]. The SMARTICLES were made under acidic conditions, resulting in protonation of CHEMS and MO-CHOL, so that the negatively charged miRNA can be efficiently encapsulated[66]. After miRNA encapsulation the pH was set to 7.5, deprotonating CHEMS and MO-CHOL and resulting in a negative charge over the vesicle membrane of -40 mV[65]. This also results in a vesicle that is not positively charged in the body, which is favourable since positively charged liposomes are linked with toxicity. Previous experiment have shown that used positively charged liposomes could for example result in hepatotoxicity, pulmonary inflammation and activation of the complement system[67]. In *vivo* experiments were done in which mice with existing tumours were injected with SMARTICLES plus miR-34a and led to a tumour regression and prolonged survival[68]. After these kind of promising results the drug got into the clinical evaluation, Phase I (see below) [69].

4.3 MRX34

MRX34 is essentially a SMARTICLE filled with double stranded miR-34a mimics, in which its active strand is identical to human miR-34a[70]. A SMARTICLE can encapsulate a high number of miRNAs (see previous paragraph) and has a long survival time in the bloodstream, which is more than 24 h after treatment[71]. These MRX34s were the first miRNA therapeutics that went into clinical trials, Phase I, results will be shown in the next paragraph.

4.3.1 Results in patients

A total of 47 patients of over 18 years that did not respond to standard treatments participated in a study employing MRX34. Tumour activity was evaluated through computer tomography (CT) or magnetic resonance imaging (MRI) scans. The concentration in the blood of the miR-34a mimic was measured with quantitative real time polymerase chain reaction (qRT-PCR). The best result was in a patient showing a partial response (PR), which means that there was a decrease in tumour diameter. Furthermore, 6 patients showed a situation of stable disease (SD), which means that the cancer did not grow nor shrink. In 32 patients a progressive disease (PD) was seen, meaning that the cancer evolved further[70]. However promising the results, the clinical trials were put on hold after 5 of the patients were diagnosed with severe immune-related adverse events[83].

4.3.2 Combination therapy

The combination of different drugs in the treatment of cancer showed some promising results. One of the advantages is reduced resistance because cancer cells have difficulty in adapting to two different drugs that have effects in different pathways. Another advantage is that combination therapy could result in less toxicity towards normal cells while it actually induces cytotoxic effects in cancer cells. This effect could be achieved by using one drug that induces cell death in both healthy and cancer cells, and another drug which is an antagonist to the first drug that is only available for healthy cells, thus will protect the healthy cells [72]. Take for example the caspase inhibitor, Z-DEVD-fmk, which is mostly effective in healthy cells since cancer cells often lack caspases and Z-DEVD-fmk is pumped out of the cancer cell because of the p-glycoprotein production in cancer cells. Use Z-DEVD-fmk in combination with flavopridol, which induces caspase dependent cell death in both cancer cells and healthy cells. This way only cancer cells will be affected by flavopridol, since the healthy cells are protected by Z-DEVD-fmk[73].

4.3.2.1 Erlotinib in non-small cell lung cancer

Erlotinib is a drug used in cancer patients which is a molecule that is directed against the epidermal growth factor receptor EGFR, inactivating this receptor. Some positive result was observed with respect to non-small cell lung cancer (NSCLC), although in some patients the cancer cells remained resistant. Applying miR-34a in addition to erlotinib resulted in a restoration of the sensitivity of the NSCLC cells to erlotinib[74].

4.3.2.2 Chemosensitivity

One of the most common strategies to cure cancer is the use of chemotherapy. However, some patients do not respond to such therapies. Recent studies have shown that elevated levels of pre-miR-34a led to cancer cells being more sensitive to chemotherapy. An unexpected finding was the increase in miR-34a expression during cisplatin-chemotherapy. This observation suggests that a combination of miR-34a therapeutics and cisplatin-chemotherapy might give better results than when each of them are used seperately[75,76].

5 Conclusion

miRNAs have been shown to be dysregulated in many types of cancers and in many other diseases and their importance in maintaining proper cell function cannot be ignored. The re-instalment of normal miRNA levels via miRNA therapeutics has already shown to be of great potential. The principle has been detailed in this thesis by using the miRNA miR-34a as an example. miR-34a is a "star" among the miRNA since it has many targets and has been shown to play a major role in cancer development. miR-34a is downregulated in many cancer cell types and employing miR-34a as a therapeutic delivered promising results both in animal model and in human studies. Also its combination with other cancer drugs resulted in a positive outcome in certain cases. Especially the observation that miR-34a can sensitize NSCLC cells to chemotherapy is a surprizing and promising effect. Notwithstanding this, the clinical trials were terminated because several patients developed severe adverse immune-related reactions towards the drug. This outcome could be seen as a huge drawback and could mean that research on miR-34a as a therapeutic is no longer valuable. However, since miR-34a does show a lot of potential future research should be focussed on understanding how this immune response comes about and how it could be minimized or removed.

The gene targets of miR-34a are mostly oncogenes and miR-34a is greatly involved in the p53 pathway. Furthermore, transcription of the miR-34a gene resulted in a clear senescence state of the cells or they even went into apoptosis. A future direction of studies would be to further investigate how miR-34a regulates these and possibly other cell processes and, thus, to get a clear picture of its network. Especially, as already mentioned above, more research has to be done on how it leads to the severe immune reaction in patients, how these immune responses were triggered and how to counteract these negative adverse events.

MiR-34a is not the only miRNA that has potential of being used as a therapeutic. Many other miRNAs have already gone into Phase II clinical trials. Some of the miRNAs are not only used in cancer treatments, they are also employed in treatments of other diseases like cardiovascular disease and atherosclerosis[77,78].

In conclusion, miRNAs show a lot of potential as therapeutics in the treatment of several diseases. Although negative result were initially obtained with miR-34a, it could still be a proper potential drug against cancer especially maybe also or specifically only in combination with other cancer drugs. A lot of research still needs to be done to get to the point that this technique will be preferentially used over the more conventional methods.

6 References

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