The potential of miRNAs in renal regeneration

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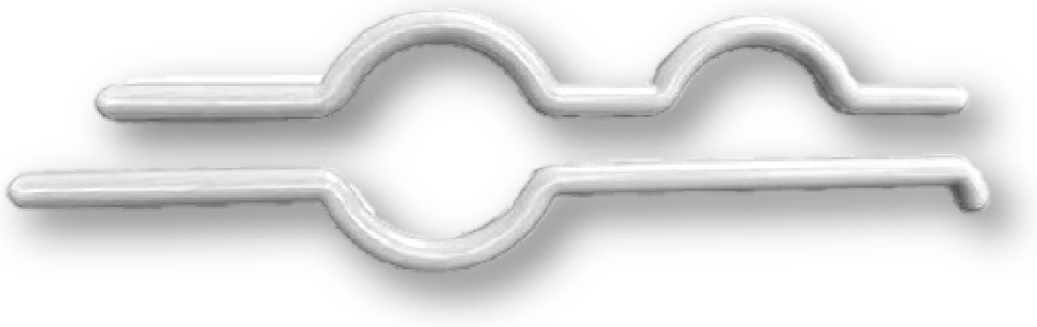


Contents

Summary

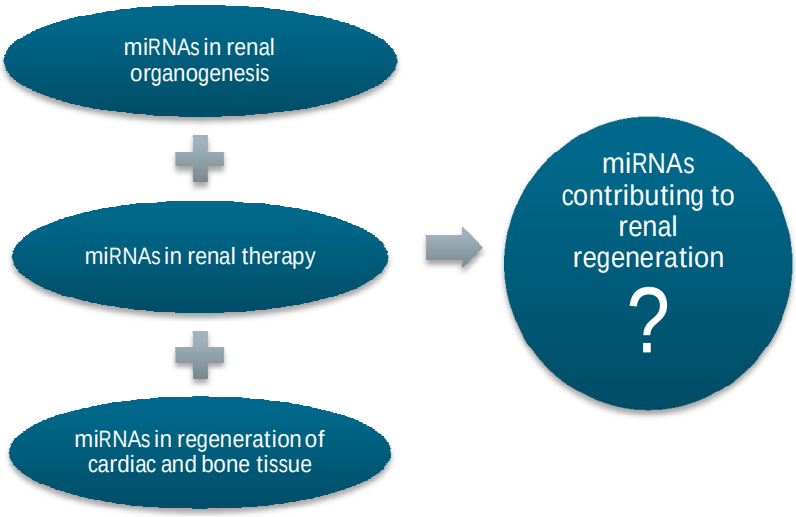
microRNAs (miRNAs) are small non-coding RNAs that regulate mRNAs at post-transcriptional level. This way, miRNAs can influence several target mRNAs and gene expression in an epigenetic fashion. Since the discovery of miRNAs, it has been found that miRNAs play a pivotal role in various organs, including the kidney. miRNAs contribute to kidney organogenesis and specific miRNAs are related to specific renal diseases. In addition, miRNAs have been examined for their potential to the regeneration of cardiac cells. It has been found that cardiac function in vivo can improve after the administration of specific miRNAs. Despite these findings, it is unclear whether those small molecules can contribute to renal regeneration as well. The aim of this thesis was to investigate whether miRNAs hold future prospects in the field of renal regenerative medicine. It was found that miRNAs hold the potential either to serve as therapeutical tools or as biomarkers, however, no studies are reported that examine regeneration in renal cells due to the application of miRNA. However, the function of miRNAs in renal disease is investigated at the moment. miRNA-192 and miRNA-21 can ameliorate fibrosis in diabetic nephropathy. Furthermore, miRNAs are necessary for tubular maturation. What is more, other strategies in renal regeneration such as organ repopulation or stem cell reprogramming evaluate renal regeneration. So far, these findings have only been demonstrated apart from each other. Therefore, it is suggested that miRNAs reported in organogenesis and disease be integrated with the above mentioned other renal regeneration strategies. Future studies that combine the aspects of cellular regeneration and fibrotic degeneration might be useful to address the question whether miRNAs can indeed help to regenerate renal function.

Introduction

The number of patients with severe kidney diseases is estimated at 60.000 in the Netherlands alone, with an estimated queue time of four years for kidney transplantation (reference 1). Unfortunately, the donor supply is not sufficient to match the patient demand (Ireland, 2012). Moreover, complications after renal transplantation remain a problem in kidney recipients (Loupy, 2012). The kidney is one of the most vital organs with multiple functions. Firstly, it regulates body fluid homeostasis; secondly, it serves as a disposal of wastes. Next, the balance of electrolytes is regulated by the kidney and the fourth important function is the secretion of various hormones such as erythropoietin (Ma, 2013). Multiple diseases result in renal dysfunction such as diabetes mellitus type 1 and 2, cancer, polycystic kidney disease (PKD) and others (Ma, 2013). Therefore, the need to develop better therapeutic interventions is high.

To date, several therapeutic mechanisms are being investigated that target the kidney, such as pharmacotherapy and kidney transplantation if the first is no longer sufficient. A more recent form of therapy involves micro-RNAs (miRNAs), short DNA fragments that modify mRNA expression profiles (Bartel, 2009). Several renal specific miRNAs have been discovered, opening new pathways in renal therapy. Firstly, it has become apparent that miRNAs have therapeutic potential in renal failure since numerous studies have reported specific miRNAs to be upregulated or downregulated related to a renal disease. The miRNAs reported to be present in diseases might be excellent targets as it comes to developing pharmacotherapy. However, this approach will only attenuate complications of the disease instead of tackling the original cause. Therefore, the development of a regenerative strategy that can overcome the original cause of a disease is the preferred option. Appropriate kidney regeneration will be a major solution, because renal transplantation remains the only curative option (Song, 2013). Secondly, miRNAs are already emerging in the field of cardiac (Brás-Rosário, 2013; Eulalio, 2012) and bone (Li, 2013) regenerative medicine. In addition, studies have been performed that describe an essential role for miRNAs in the organogenesis of the kidney (Patel, 2012; Ma, 2013). Despite these findings, the role of miRNAs in renal tissue regeneration remains unclear. The current situation in renal regenerative medicine can be summarized by figure 1, because miRNAs in relation to the kidney are studies from three different points of view.

Figure 1 – To date, miRNAs have been studied from different points of view. However, it remains unclear whether miRNAs have significant relevance for the field of renal regenerative medicine. This thesis will investigate the potential of miRNAs in the latter field.



Therefore, this review will examine the regenerative prospects of miRNA in renal diseases. The miRNA contribution to renal therapy is investigated by an analysis of the current knowledge on miRNAs in renal diseases and miRNA studies in regenerative medicine focusing on other organs. To date, such an approach has not been described.

miRNA mechanism of action

Epigenetics is described as modifications that influence proteins downstream DNA without manipulating the original DNA sequence. These modifications can be inherited during cellular proliferation (Feinberg, 2004). The three major epigenetic mechanisms are DNA methylation, histone modification and miRNA expression. miRNAs were observed for the first time in C. elegans (Lee, 1993). miRNAs are endogenous small non-coding RNAs (20-22 nucleotides) that control gene expression at post-transcriptional level (Kato, 2012). After the discovery of miRNAs, these small molecules have been found in multiple species and in multiple organs (Bartel, 2009). Biogenesis of miRNAs occurs in the nucleus as primary miRNAs, where after the latter are processed to pre-miRNAs by an enzyme called Drosha (Figure 2). Next, exportin5 facilitates pre-miRNA transport from the nucleus to the cytoplasm. Here, an enzyme called Dicer forms the final miRNA product (Kato, 2012). Here, the miRNA is incorporated in the RNA-induced silencing complex (RISC) (Chandrasekaran, 2012). Generally, nucleotide 2-7 bind to the 3' untranslated region of the target mRNA, these miRNA nucleotides are called the seed sequence (Figure 2) (Gregory, 2008). Remarkably, miRNAs can modulate multiple mRNAs and vice versa, one mRNA can be modulated by multiple miRNAs (Shenouda, 2009). This way, miRNAs serve multiple purposes such as cellular differentiation and proliferation control (Brás-Rosário, 2013).

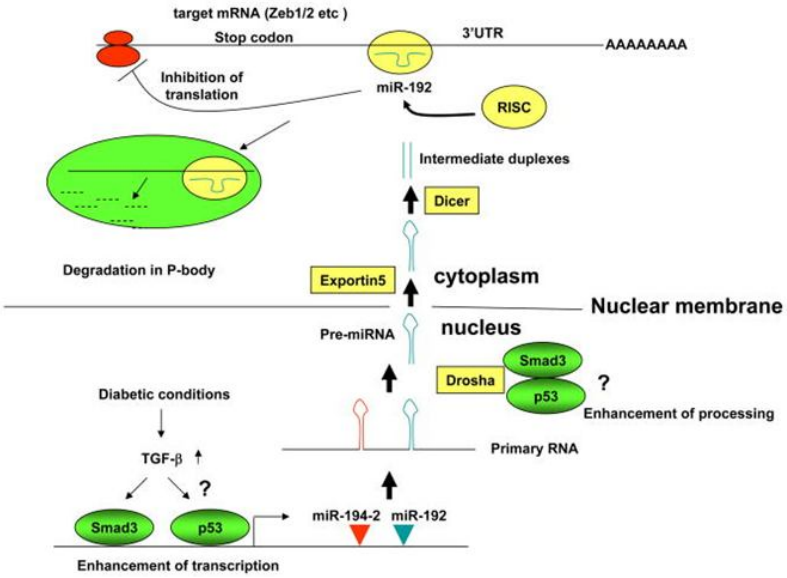


Figure 2 – miRNA mechanism of action. First, primary RNA is synthesized in the nucleus of the cell. In the next step, Drosha cuts primary RNAs in pre-miRNAs. Exportin-5 facilitates pre-miRNA transport from the nucleus to the cytoplasm. Hereafter, the enzyme Dicer is involved in finishing the miRNA product. The complete miRNA product binds the 3' untranslated region at the messenger RNA. From: Kato, 2012.

Inactivation of the enzymes Dicer and Drosha has led to incomplete renal organogenesis. Evidently, miRNAs are crucial in this process (Patel, 2012; Ma, 2013). One of the most striking features is the reversible character of epigenetic modulations (Henrique, 2012). This contributes to the increasing interest in miRNA research.

Various miRNAs have the ability to downregulate fibrosis-associated genes in different organs such as heart and kidney; this will be discussed further in the following sections. Myofibroblasts play a key role in the onset of chronic kidney disease, because they can contribute to the formation of extracellular matrix proteins. What is more, myofibroblasts are involved in fibrotic situations and can therefore contribute to organ dysfunction as well (Hinz, 2012). In addition, miRNAs might fulfill a role as biomarkers due to their non-invasive measurement options and physiological stability. Therefore, identification of miRNAs downstream of renal diseases might be useful in screening and eventually in the earliest possible state of disease (Kato, 2012).

miRNAs in renal diseases

To gather more insights in the role of miRNAs in renal disease, multiple studies have been performed that examined human tissue samples and animal models. A wide range of predominant kidney diseases has been investigated for the presence of specific miRNAs such as diabetic nephropathy, renal cell carcinoma, polycystic kidney disease, acute kidney injury, lupus nephritis and IgA nephropathy (Ho, 2013). An overview of the miRNAs reported in renal diseases so far is given by figure 3. It is shown that both upregulation and downregulation of miRNAs can mediate a downstream target, indicating the complexity of miRNA networks. This thesis will mainly consider miRNA involvement in diabetic nephropathy and renal cell cancer, due to the fact that characteristics involved in fibrosis and cell proliferation are of main interest if it comes to renal regeneration. One of the cell types found to be involved in renal diseases is the renal pericyte, which plays a role in homeostasis and responses to injury. This type of cell has been described in various other organs such as skin, lungs and central nervous system to contribute significantly to extracellular matrix protein accumulation (Ren, 2013). In the kidney, those cells contribute to extracellular matrix production especially in the interstitial space of tubules and capillaries. It was found that miRNA-21 is highly upregulated in pericytes by silencing peroxisome proliferator activated receptor alpha (PPAR-α) and fatty acid oxidation in mitochondria. The PPAR-α receptor is involved in lipid metabolism. As a consequence, reactive oxygen species are increased and energy production is decreased, resulting in the promotion of renal fibrosis (Ren, 2013; Chau, 2012). Repression of miRNA-21 expression resulted in improved results for fibrosis formation (Chau, 2012).

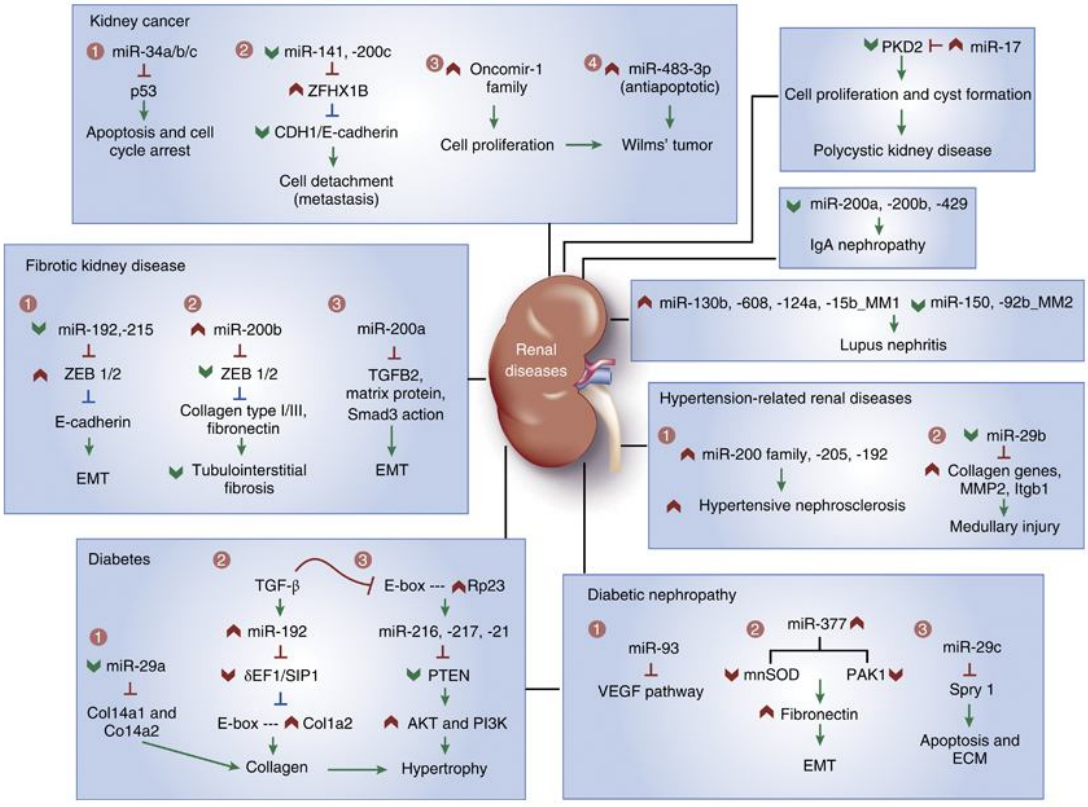


Figure 3 – overview of miRNAs involved in kidney diseases. miRNAs that are highly correlated to one specific disease can either function as a biomarker or as a target for pharmacotherapy. If it comes to renal regeneration, miRNAs involved in fibrotic kidney disease are of particular interest. From: Chandrasekaran, 2012.

Diabetes

One of the major complications in diabetes is diabetic nephropathy (DN). Characteristics include excessive collagen disposition and extracellular matrix accumulation, glomerular and tubular hypertrophy (Ma, 2013; Chandrasekaran, 2012), and also glomerular basement membrane thickening (Putta, 2012), resulting in severe kidney injury. In addition, it is the most important cause of end-stage renal disease and is associated with high mortality and morbidity (Chandrasekaran, 2012). Unfortunately, the exact cause of DN is not yet understood.

One of the key mediators in DN is transforming growth factor beta (TGF-β) (Kato, 2012). TGF- β mediates the expression of miRNA in DN, however it has several other purposes. This way, it is worth the while to investigate miRNAs downstream TGF- β that might be more specific and as a consequence have fewer side effects (Kato, 2012). TGF- β is involved in the accumulation of extracellular matrix and renal hypertrophy, because it can bind to E-box repressors that normally repress the transcription of several genes coding for collagen and cadherin synthesis (Kato, 2012). In particular, ZEBI and ZEB2 are involved that normally repress the collagen type 1 alpha 2 (Col1a2) gene (Kato, 2012). miRNA-192 is reported to bind to the ZEB E-box repressors, preventing the binding of these repressors to their mRNA targets. This way, Col1a2 expression is upregulated and contributes to fibrotic conditions (Kato, 2012). miRNA-192 is especially of interest because it is highly expressed in the kidney and is increased by TGF- β (Kato, 2012).

In addition to miRNA-192, other miRNAs are identified in the kidney (Figure 3). Also, miRNA-21 knock-down plasmids are reported to improve renal function in diabetic mice and might therefore be a good target for new therapeutic strategies. Db/db mice showed increased levels of miRNA-21 compared to wildtype mice and suppression of this miRNA resulted in improvements in the progression of diabetic nephropathy (Zhong, 2013). *In vivo* inhibition of miRNA-21 ameliorated renal function in diabetes type 2 mice (Zhong, 2013). Another miRNA that has proved its positive effect on renal fibrosis is miRNA200b, by decreasing fibronection, collagen I and collagen III levels. As a consequence, tubulointerstitial fibrosis was ameliorated (Oba, 2010; Chandrasekaran, 2012). In addition, miRNA-200a and miRNA-141 are essential in the development of TGF-β dependent renal fibrosis (Chadrasekaran, 2012).

However, the functional role of miRNA is not yet examined in the kidney. To further develop the therapy, this must be clarified (Zhong, 2013). Moreover, it was found that downregulation of miRNA-192 significantly ameliorated both renal fibrosis and proteinuria *in vivo* in mice (Putta, 2012). Together, these data demonstrate the important role of miRNA-192 in diabetic mice, therefore additional research is needed to develop a successful therapy.

Renal cell cancer

Of all renal cancer types, renal cell cancer (RCC) is the most predominant in adults (Chow, 2010 in Ma, 2013). Epigenetic regulation in cancer cells is frequently disturbed compared to normal cells (Henrique, 2012). It was pointed out that miRNA dysregulation is involved in renal cell cancer (Chandrasekaran, 2012).

Firstly, a miRNA expressed in RCC is miRNA-99a. Experiments *in vitro* and *in vivo* have shown that this miRNA is increased in human RCC tissue compared to non-RCC tissue and that restoration of this miRNA in cell lines level helps to stop cellular proliferation *in vitro* (Cui, 2012). Other miRNAs are involved in intracellular pathways and can therefore either stimulate apoptosis or cellular proliferation. It was found that miRNA-141, a member of the miRNA-200 family, is downregulated in human RCC patient samples compared to samples of healthy people. In addition to this finding, it was also reported that re-expression of this miRNA led to cell proliferation in cell cultures (Yu, 2013). Another study confirmed that miRNA-141, together with other miRNA-200 family members, is downregulated in RCC human samples (Yoshino, 2013).

It was found that members of this family play an essential role in the onset of epithelial-to-mesenchymal transition (EMT), a process that contributes to the onset of tumor metastasis. The miRNA-200 family is known to target mRNAs downstream the ZEB1 and SIB1 gene, responsible for the repression of epithelial marker E-cadherin. Constitutive expression of these miRNAs prevented canine epithelial cells exposed to TGF-β to undergo EMT, indicating that downregulation of this miRNA family is critical in the onset of this process (Gregory, 2008). EMT is not only reported as a process in the onset of RCC, it also contributes to tissue remodeling in embryonic development (Gregory, 2008). On the other hand, it was found that miRNA-141 can repress TGF- β expression in vitro (Wang, 2010). This suggests another pathway for miRNA-141 to inhibit EMT.

Overall, miRNAs play key roles in various renal diseases as described above. Interestingly, miRNAs can be both protective and pathologic, due to the fact that miRNA-192 and miRNA-141 demonstrated an improved situation after upregulation, whereas in the case of miRNA-21 the opposite is true. Secondly, miRNAs have therapeutical potential in another perspective. Renal cell cancer has a high mortality rate due to late diagnosis, therefore miRNAs as diagnostic biomarkers can come in advance especially for this disease. Several serum miRNAs are being investigated as RCC biomarker (Ma, 2013). Also, miRNAs might help in the classification of renal cancer subtypes (Spector, 2013). Another miRNA that has promise to function as biomarker is miRNA-210, which is reported to be upregulated in blood plasma of patients with acute kidney injury (Ma, 2013). This disease is not further discussed in this thesis, however, this finding indicates another therapeutic value for miRNAs in renal diseases. This contribution of miRNAs seems on the verge of therapeutic application, because more and more miRNAs are discovered to be specific for a disease or tumor, and miRNA collection can be done in a non-invasive way.

miRNAs in renal development

Several cellular processes contribute collectively to the organogenesis of the kidney, such as mesenchymal-to-epithelial transition, epithelial cell polarization and branching morphogenesis. Development of the kidney has origin in the mesodermal region, where the ureteric bud induces mesodermal cells to form nephron progenitors (Ma, 2013). These cells either form new nephron progenitors or differentiate in various nephron celtypes. Hereafter, the ureteric bud forms the collecting ducts (Ho, 2013). At this point, mammals differ from their anchestors. After renal injury, nephron neogenesis does not occur in mammals; however it has been reported in other animals such as fish. A major reason for this difference might be the evolutionary implementation of Henle's loop that overcame the gap of living on land rather than in an aqueous environment. Other renal cells such as glomerular and tubular cells can be regenerated in adult life due to appropriate progenitor cells; however this is not the case for Henle's loop (Romagnani, 2013). Therefore, the regenerative capacity of the kidney remains limited.

Experiments in which the modifying enzyme Dicer is knocked down gain insights in the contribution of miRNAs to kidney development. For example, Dicer knockdown in renal progenitors results in a decreased number of progenitors and eventually in a decreased nephron number (Nagalakshimi, 2011; Ho, 2013). In addition to this, it was estimated that miRNAs are essential in tubule maturation (Patel, 2012). Deletion of the enzyme Dicer resulted in cyst formation in 75% of Dicer mutant mice. Dicer mutations were chosen in such a way that only renal tubule maturation was affected and not renal vesicle formation, this modification allowed for examining renal maturation in a final stadium specifically. Also, mutant mice showed higher mortality rates compared to the control group (Patel, 2012). After miRNA microarray analysis it was found that especially miRNA-200 family members were downregulated after deletion of Dicer (Patel, 2012). Furthermore, especially miRNA-30 is important in renal development (Ma, 2013). It is found that knockdown of miRNA-30-a-5p resulted in defective development of the pronephros, an organ in the species Xenopus analogue to the kidney in human (Ho, 2013). However, a complete view of the contribution of miRNAs in this process remains to be clarified (Ho, 2013).

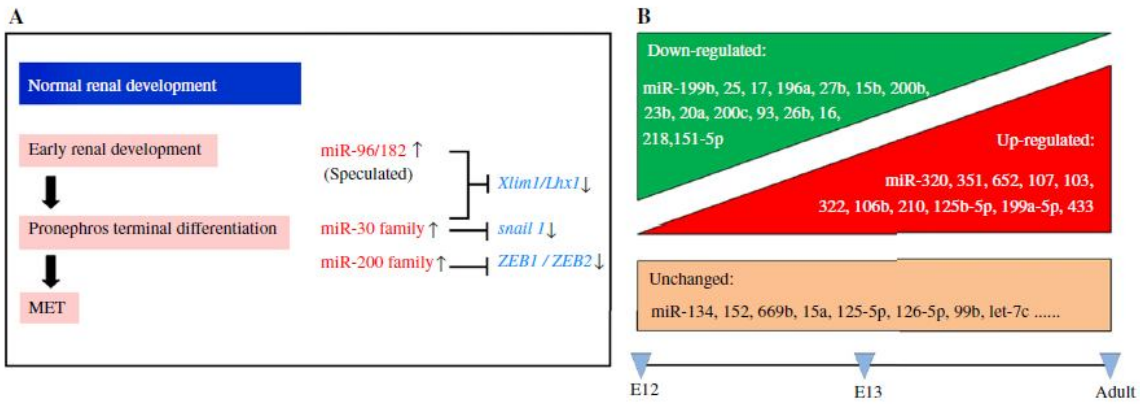


Figure 4 – overview of miRNAs that play a crucial role in normal renal development. A: a schematic overview of miRNAs which are important for pronephros formation in the Xenopus species. B: miRNAs involved in mouse kidney organogenesis. miRNAs can either play an important role at the beginning or later on in the developmental process. From: Ma et al., 2013.

miRNAs in organ regeneration

Despite the use of miRNAs in renal disease therapy, several papers report miRNAs that play an active role in cell proliferation (Eulalio, 2012). This part is of main interest as it comes to renal regeneration. Papers describing the application of specific miRNAs that facilitate renal cellular proliferation could not be found, however, such reports on other organs than kidney do.

Cardiac regeneration

One of the best characterized examples is cardiac regeneration. It goes without saying that the heart and kidney cannot be compared parallel as it comes to regeneration; however, the pathological accumulation of extracellular matrix is seen in both organs. Similar to the kidney, fibrosis occurs in the heart as a response to injury and repair of adult cells is a problem. It is reported that miRNAs have the capacity to induce cardiac regeneration (Eulalio, 2012). Another resemblance is the dual application of miRNAs, first the link of specific miRNAs to disease and secondly the involvement of miRNAs in cellular proliferation and organogenesis (Karakikes, 2013).

Firstly, several miRNAs from a miRNA library were selected in an experiment in which cardiomyocyte cell cultures received different miRNA subtypes and were stained for DNA synthesis markers. The 10 miRNAs that increased these proliferation markers the most were selected for following experiments. It was pointed out that histone H3 phosphorylation, a marker for late mitosis in cardiomyocytes, was increased after treatment with the earlier miRNA selection in rat and mouse cardiomyocytes (Eulalio, 2012). In another experiment, the examination of cell cycle re-entry revealed that a selection of miRNAs (miRNA-67, has-miRNA-590-3p and has-miRNA-199a-3p) increased cardiomyocyte proliferation as well (Eulalio, 2012). To investigate whether these findings contribute to cardiac regeneration, the capacity of cardiac function was tested afterwards. Mice underwent a cardiac infarct, were treated with the above mentioned miRNAs and showed ameliorated left ventricular ejection fraction (Eulalio, 2012).

In addition, another miRNA has been investigated for the treatment of cardiac remodeling (Karakikes, 2013). It was reported that miRNA-1 therapy ameliorates hypertrophy progression in rats. Rats received either aortic banding or sham operations and were randomly chosen to receive miRNA-1 or control vector via tail-vein injection (Karakikes, 2013). Compared to the previous study of Eulalio, in which animals received a cardiac injection, this miRNA delivery strategy is a better approach to clinical practice. Seven weeks after the aortic banding operation, animals that received the miRNA-I vector showed significant improvements in left ventricular wall thickness compared to control animals. This study demonstrated that cardiac hypertrophy could be partially reduced (Karakikes, 2013). The fact that miRNA delivery has provided its effect in remodeling reversal (Karakikes, 2013) indicates that further studies in this field might be worthwhile in the kidney as well. In a third study, it was demonstrated that miRNAs are capable of mediating the direct converstion of fibroblast to cardiac myocytes (Jayawardena, 2012). Similar to experiments performed in the study of Eulalio, a selection of miRNAs and miRNA combinations was done to filter the combinations with the greatest potential to induce a phenotypic shift. It was found that cardiac fibroblasts displayed cardiac myocyte markers after exposure to miRNAs (Jayawardena, 2012).

Bone regeneration

miRNAs have also been established as potential contributors to bone regenerative medicine (Li, 2013). Similar to cardiac studies previously mentioned, an analysis of the miRNA expression profile was performed to examine which miRNAs would be the most useful for further experiments. miRNA-26 was found to be important in this test and was therefore administrated to mice which underwent a 5mm bone defect (Li, 2013). Neo-formed bone was significantly increased in the miRNA-26 treated group compared to control animals (Li, 2013).

Collectively, these studies provide evidence that miRNAs have great potential in mice. It should be taken into account that the current data are not yet translated into a human model and thus might have other effects in that situation. However, if it comes to kidney regeneration, other strategies are concerned as well. It was stated that progress in the induction of renal cell lineages is especially hampered by the multiple cell types present in the kidney (Morizane, 2013). One example is regeneration of the adult kidney by inducing embryonic stem cells toward renal tubular cells (Morizane, 2013). Embryonic cells were exposed to a medium with different factors, for example a protein called activin, that play a role in the induction of kidney specific protein expression. In contrast to complete regeneration of an organ, Song et al. demonstrated the potential of decellularized cadaveric kidneys repopularized with endothelial and epithelial cells (Song, 2013). It was established that this decellularization process did not harm the vascular, glomerular and tubular structures. This architecture is essential for the kidney to perform filtration, absorption and secretion processes (Song, 2013). In contrast to miRNAs implementation in renal pharmacotherapy, this report proposes a strategy that can replace an entire kidney and might therefore be more effective.

Discussion

In the last few years, several research groups have reported that pro- and anti-pathological miRNAs are involved in the kidney. miRNAs can influence cellular differentiation, therefore this combination holds potential for the use of miRNAs in renal regenerative medicine. miRNA-192 contributes to DN (Putta, 2012) and miRNA-21 plays a role in the onset of fibrosis (Zhong, 2013), whereas miRNA-30 is involved in renal organogenesis (Ma, 2013). The first step in overcoming DN is the prevention or destruction of the accumulated extracellular matrix, which ideally will be followed by a regeneration step that stimulates the formation of new nephrons. Several miRNAs have been examined separately, but not together in one and the same study. The combination of the stimulation of cellular proliferation and fibrotic destruction might result in a model that improves renal function. Future studies with a design that involves multiple miRNAs might enlighten insights on the contribution of miRNAs especially for regeneration capacity.

A similar study design is implemented in research on bone and cardiac miRNA experiments, as mentioned above, these studies revealed either single miRNAs or miRNA combinations that contribute to cellular proliferation (Li, 2013; Eulalio, 2012; Jawayardena, 2012). However, to date no such reports exist and it is therefore still poorly understood whether miRNAs can contribute to renal regeneration. Another point worth mentioning, papers reporting negative results concerning miRNAs in renal regeneration could not be found. This can either indicate that such approaches have not been performed or that small studies with negative results have not been published because they were not worth the while.

Different research groups have found similar evidence to support that specific miRNAs belong to specific pathologies. For example, miRNA-192 has been reported by multiple research groups to be involved in fibrosis (Putta, 2013; Kato, 2012). On the other hand, the miRNA-200 family is reported to have different influences in the kidney. This family is reported to influence the PKD-gene on the one hand (Patel, 2012), whereas on the other hand, this miRNA family is mainly involved in transcriptional repressors ZEB1 and SIP1 (Gregory, 2008). Both studies highlight another role for the miRNA-200 family in the kidney rather than contradict each other, this shows that the knowledge on miRNAs in the kidney remains to be elucidated further, as stated earlier (Ho, 2013). Furthermore, the pleiotropic character of miRNAs is reported both as advantage and as drawback. Because of this pleiotropic effect, an entire pathway is affected instead of a single gene or protein, and this might be beneficial because the effect of miRNA modification can be great (Tsongalis, 2013). For example, this is the case with miRNA-192 in diabetic nephropathy. Kato et al. argue this as an advantage, whereas Putta et al. consider it challenging because miRNA-192 can have other targets as well that are still unknown.

A proposed scheme for the application of miRNAs in clinical practice is shown in figure 5. miRNA might serve multiple purposes in the future such as a diagnostic biomarker that can be detected in blood or urine. On the other hand, miRNAs can fulfill a role in pharmacotherapy as miRNA inhibitor or anti-miRNA, depending on whether a miRNA should be inhibited or upregulated to improve a disease state (Cui, 2012). However, the potential for miRNAs in the field of regenerative medicine is not taken into account. As mentioned above, different studies considering cardiac regeneration have experimented with the combination of different miRNAs and their effect in cellular proliferation. Currently, such studies could not be found for the kidney. Therefore, this strategy might be valuable to detect another miRNA application that can enhance figure 5.

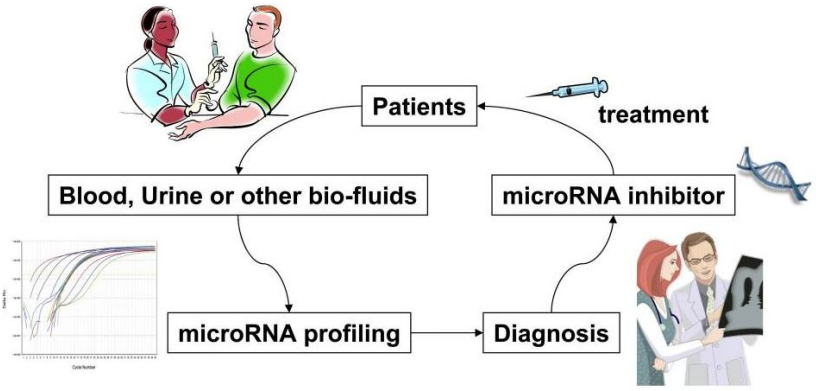


Figure 5 – Role of miRNA in diagnosis and treatment of renal disease in the ideal situation. Both therapeutic interventions and diagnosis of diseases are events in which miRNAs can play a beneficial role. From: Kato, 2012.

Despite the potential of miRNAs so far, some hurdles remain. As far as miRNA pharmacotherapy is concerned, additional studies are strictly required to identify the appropriate clinical implementations. miRNAs have a pleiotropic character and the need is high to perform an extensive side effect study before miRNAs can be officially considered as therapeutically applicable. This requires a careful validation which remains a great challenge. What is more, miRNA delivery must be optimized before it can be used as regeneration tools (Li, 2013). One of the gaps that must be overcome before these findings can be implemented in practice is the administration of miRNA. Several miRNA delivery strategies have been developed, such as viral vectors or liposomes (Pereira, 2013). miRNA-1 has been delivered to hypertrophic rats by a viral vector. The fact that miRNAs are not only uprising in the field of renal medicine, but also in other organs might help to accelerate the insights in the acquirement of optimal miRNA delivery techniques. However, delivery of nucleic acids to the appropriate target sites can be a problem because those molecules can be degraded and inactivated before they reach their target (Ma, 2013).

Conclusion

To conclude, miRNAs promise to be useful as therapeutic interventions in kidney diseases on the one hand, as well as diagnostic biomarkers on the other hand (Cui, 2012). It can be stated that different strategies can contribute to the same goal that is ameliorating renal diseases (Figure 6). This way, an increase of miRNAs in urine or blood can serve to detect the onset of a particular disease. Furthermore, miRNAs involved in the pathogenesis of renal diseases is currently being investigated. More and more miRNAs are reported to be involved in diabetic nephropathy, renal cell cancer or other renal diseases. miRNAs involved in renal cell proliferation, extracellular matrix accumulation or fibrosis can be analyzed for regenerative potential in renal cells in future studies. The fact that several miRNA studies have proven their beneficial effect in mice cardiac tissue suggest that such studies in renal tissue could be performed as well, because both organs suffer from fibrosis and extracellular matrix accumulation after injury. The regenerative application might be optimized by a combination of miRNA tools and other regenerative strategies, such as the repopularization of an adult kidney or the application of miRNAs in stem cells (Song, 2013; Morizane, 2013). In addition, the combination of miRNAs involved in the onset of fibrosis and in the organogenesis of the kidney could be considered together.

Figure 6 – summary

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