MikroRNA u kroničnoj bubrežnoj bolesti i zatajivanju srca

MicroRNA in Chronic Kidney Disease and Heart Failure

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SAŽETAK: MikroRNA (miRNA) male su nekodirajuće jednolančane RNA molekule građene od 21 do 25 nukleotida koje reguliraju ekspresiju gena na posttranskripcijskoj razini. Više od tisuću različitih miRNA kodirano je ljudskim genomom. Posljednjih godina za miRNA pokazalo se da su uključene u različite biološke procese poput diferencijacije, proliferacije i apoptoze stanica. U patogenezi brojnih bolesti u čovjeka jest miRNA, primjerice pri zatajivanju srca, šećernoj bolesti, pretilosti, bubrežnim, zaraznim i malignim bolestima te genskim poremećajima. Brojni dokazi govore u prilog važnosti miRNA u razvoju bubrega i fiziologije srca. Stoga nije iznenađujuće da se poremećaj regulacije miRNA može uočiti u različitim bubrežnim i srčanim bolestima. Otkriće da cirkulirajuće miRNA mogu biti detektirane u serumu i plazmi te da je njihova ekspresija može varirati zbog bolesti značajan potencijal njihove uporabe kao novog biomarkera. Terapija temeljena na miRNA može djelovati ili obnavljanjem njihove funkcije ili blokiranjem njihove ekspresije i aktivnosti, zbog čega je obećavajuća.

SUMMARY: MicroRNA (miRNA) are small non-coding single-strand RNA molecules built from 21-25 nucleotides that regulate gene expression at the post-transcription level. More than a thousand different miRNA are coded in the human genome. During the last few years, it has been found that miRNA are involved in different biological processes such as differentiation, proliferation, and apoptosis of cells. miRNA is part of the pathogenesis of many diseases in humans, such as heart failure, diabetes, obesity, kidney, infectious and malign diseases, and genetic disorders. Much evidence speaks in favor of the significance of miRNA in the development of the kidneys and physiology of the heart. It is thus not surprising that a disorder of miRNA regulation can be observed in many different kidney and heart diseases. The discovery that circulating miRNA can be detected in the serum and plasma and that their expression can vary due to disease represents a significant potential for their use as a new biomarker. Therapy based on miRNA can act by either restoring their function or blocking their expression and activity, making it very promising.

KLJUČNE RIJEČI: mikroRNA, kronična bubrežna bolest, zatajivanje srca.

KEYWORDS: microRNAs, chronic kidney disease, heart failure.

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MikroRNA (miRNA) male su nekodirajuće jednolančane RNA molekule građene od 21 do 25 nukleotida koje reguliraju ekspresiju gena na posttranskripcijskoj razini. MiRNA se vežu za mRNA različitih gena, što uzrokuje njihovu degradaciju[1,2]. Stoga miRNA imaju važnu ulogu u regulaciji gotovo svakoga staničnog procesa, a promjena u njihovoj ekspresiji uzrokuje određena patološka stanja. Zbog toga miRNA postaju jedan od najvažnijih fokusa istraživanja u molekularnoj biologiji[2,3].

Sinteza miRNA počinje transkripcijom primarne miRNA (**pri-miRNA**) uz djelovanje enzima RNA polimeraze II. ili III. Sljedeći je korak

MicroRNA (miRNA) are small non-coding single-strand RNA molecules built from 21-25 nucleotides that regulate gene expression at the post-transcription level. miRNA bond to mRNA of different genes, leading to their degradation[1,2]. Therefore, miRNA have an important role in the regulation of almost any cellular process, and changes in their expression lead to certain pathological conditions. miRNA have thus become one of the most important focal points in molecular biology research[2,3].

The synthesis of miRNA begins with the transcription of primary miRNA (**pri-miRNA**) with the action of the RNA polymerase II or III enzymes.

cijepanje pri-miRNA, pri čemu nastaje sekundarni prekursor (pre-miRNA), a obavlja se s pomoću kompleksa koji se sastoji od dvaju proteina: *Drosha*, RNA nukleaze nalik na protein i njezina kofaktora *Pasha* ili DGCR8 (engl. *DiGeorge critical region 8*). *Pasha* prepoznaje i ulazi u interakciju s pri-miRNA strukturama te služi kao vodič do mjesta cijepanja, a *Drosha* tada cijepa pri-miRNA do pre-miRNA[2-4]. **Pre-miRNA** izlazi u citoplazmu, gdje se djelovanjem druge RNA-ze pod imenom Dicer cijepa do 21 – 25 nukleotida dugih dvolančanih miRNA. Dvolančana miRNA građena je od lanca vodiča koji sadržava sekvenciju komplementarnu ciljnoj molekuli i lanca putnika koji će se razgraditi[2,4]. **Zrela miRNA** integrira se u **RNA inducirani utišavajući kompleks** (engl. *RNA-induced silencing complex*; RISC) koji se tada naziva miRNA-induciranim utišavajućim kompleksom (miRISC) ili mikro-ribonukleoproteinima (miRNP). Najvažnija i najbolje karakterizirana komponenta miRISC-a jesu proteini koji pripadaju obitelji Argonaute (Ago), a u sisavaca postoje četiri takva proteina koje označujemo s Ago1-Ago4. Uloga je miRNA komplementarno vezanje s ciljnim molekulama, a Ago destabilizira, degradira ili inhibira translaciju ciljne nukleotidne sekvencije[2-4].

Nomenklatura mikroRNA

Prefiksi *mir* i *miR* rabe se za prekursore i zrele miRNA molekule. Prefiks od 3 ili 4 slova uporabljuje se da bi se označila pripadnost miRNA određenoj vrsti, npr. prefiks *hsa* (engl. *hsa* od *Homo sapiens*) označuje da je riječ o humanoj miRNA. Istovjetne miRNA koje potječu iz istoga genskog lokusa imaju isti numerički sufiks, a razlikuju se po dodatnom slovu koje im se dodjeljuje, npr. miR-10a i miR-10b2.

Klinička važnost mikroRNA

Postoji širok spektar potencijalnih kliničkih primjena miRNA molekula. MiRNA predočuju inovativne biomarkere koji bi bili neinvazivni, pouzdani, visokoosjetljivi i visokospecifični, a služili bi za postavljanje dijagnoze i procjenu prognozne različitih bolesti. Potencijalna uloga miRNA jest i u distinkciji pojedinih etioloških entiteta i podtipova bolesti. Brojna istraživanja pokazuju da miRNA sudjeluju u patogenezi raznovrsnih bolesti te bi mogle poslužiti u boljem razumijevanju patofizioloških procesa. MiRNA se pokazala kao koristan alat u odabiru vrste i intenziteta terapije, kao i u praćenju terapijskog ishoda, a i sama može poslužiti kao terapijska meta.

U budućnosti bi miRNA mogle unaprijediti personalizirani pristup pacijentu.[2,5,6]

Terapijska uloga mikroRNA

Posebna obilježja miRNA te njihova uloga u patogenezi bolesti upućuju na mogućnost važne uloge u liječenju mnogih bolesti poput ishemije miokarda, jetrenih bolesti, fibroze, inflamatornih bolesti poput reumatoidnog artritisa te malignih bolesti[7-9]. U terapijskome smislu moglo bi se djelovati ili obnavljanjem funkcije nedostatno eksprimirane miRNA ili inhibicijom prekomjerno eksprimirane miRNA[10,11].

Obnavljanje funkcije nedostatno eksprimirane mikroRNA može se izvesti na dva načina, s pomoću miRNA imitacija (engl. *miRNA mimics*) ili preekspresijom s pomoću virusnih vektora. MiRNA imitacije dvolančane su molekule koje poput endogene miRNA sadržavaju dva lanca, od kojih jedan služi

The next step is the splicing of pri-miRNA, creating the secondary precursor (pre-miRNA), and it takes place with the help of a complex consisting of two proteins: *Drosha*, a RNA nuclease-like protein and its co-factor *Pasha* or DiGeorge critical region 8 (DGCR8). *Pasha* is recognized by and interacts with pri-miRNA structures and serves a guide to the splicing location, and *Dosha* then splices pri-miRNA to pre-miRNA[2-4]. **Pre-miRNA** exits into the cytoplasm, where it is cleaved into 21-25 nucleotide-long double-stranded miRNA under the influence of a different RNase named Dicer. Double-stranded miRNA is built from a guide strand that contains the sequence complementary to the target molecule and the passenger strand that will be removed[2,4]. **Mature miRNA** is integrated into the RNA-induced silencing complex (RISC) which is then called the miRNA-induced silencing complex (miRISC) or micro-ribonucleoproteins (miRNP). The most important and best characterized component of miRISC are proteins belonging to the Argonaute (Ago) family, and there are four such proteins in mammals, designated Ago1-Ago4. The role of miRNA is complementary bonding with target molecules, and Ago destabilized, degrades, or inhibits the translation of the target nucleotide sequence[2-4].

MicroRNA nomenclature

The prefixes "mir" and "miR" are used for precursor and mature miRNA molecules. A prefix of 3 or 4 letters is used to classify miRNA as belonging to a specific species, i.e. the "*hsa*" (from *Homo sapiens*) prefix denotes human miRNA. Identical miRNA stemming from a gene locus have the same numerical suffix and differ in the additional letter assigned to them, for instance "miR-10a" and "miR-10b"[2].

Clinical significance of microRNA

There is a wide spectrum of potential clinical applications for the miRNA molecules themselves. miRNA represent innovative biomarkers that would be non-invasive, reliable, highly sensitive, and highly specific, and would serve to establish the diagnosis and evaluate the prognosis in various types of diseases. A potential role of miRNA is also discrimination between individual etiological entities and disease subtypes. Numerous studies show that miRNA participates in the pathogenesis of various diseases and could facilitate better understanding of pathophysiological processes. miRNA has shown itself a useful tool in choosing the type and intensity of treatment as well as for monitoring treatment outcomes, and can itself serve as a treatment target.

In the future, miRNA could improve the personalized approach to patients.[2,5,6]

The role of microRNA in treatment

The special characteristics of miRNA and their role in disease pathogenesis indicate a possibility of them having a significant role in the treatment of many diseases such as myocardial ischemia, liver diseases, fibrosis, inflammatory diseases such as rheumatoid arthritis, and malignant diseases[7-9]. The treatment could act by either restoring the function of insufficiently expressed miRNA or by inhibiting overexpressed miRNA[10,11].

Restoring function of insufficiently expressed miRNA can be performed in two ways, either by miRNA mimics or through pre-expression with viral vectors. miRNA mimics are double-

kao lanac vodič, a drugi kao lanac putnik. Lanac vodič sadržava genetski kôd istovjetan onomu koji sadržava ciljna miRNA molekula. Lanac putnik veže se za različite molekule, npr. za kolesterol, što olakšava unos cijele miRNA imitacije u pojedine stanice. Lažna miRNA tada se veže u RISC kompleks i na taj način nastaje miRISC koji djeluje poput onog sintetiziranog uz endogenu miRNA[7,10,12].

Inhibicija prekomjerno eksprimirane miRNA može se izvesti s pomoću miRNA spužve ili jednolančanim antisense oligonukleotidima nazvanima antimiR. MiRNA spužve koriste se transgenetskom prekomjerno eksprimiranom RNA koja sadržava komplementarna vezna mjesta s ponavljanjima za ciljne miRNA molekule koje treba blokirati. Za razliku od miRNA imitacija (lažna miRNA ili engl. *miRNA mimics*), antimiRi jednolančane su molekule komplementarne ciljnoj miRNA molekuli, kemijski modificirane da bi se ostvarili afinitet vezanja, biostabilnost i farmakokinetička svojstva. Dolaskom u stanicu antimiRi se vežu za ciljnu miRNA i inhibiraju njezinu funkciju[7,12].

MikroRNA u kroničnoj bubrežnoj bolesti

Kronična bubrežna bolest (KBB) klinički je sindrom obilježen progresivnim i trajnim oštećenjem funkcija bubrega – ekskrecijske, endokrine i metaboličke[13,14]. Incidencija bolesnika sa završnom fazom KBB-a je u porastu. U većini zemalja procijenjena prevalencija KKB-a iznosi 5 – 14 %, a raste i do nekoliko puta u starijoj životnoj dobi[15]. Prema podatcima Centralnog zdravstvenog informacijskog sustava Hrvatske i baze hospitalizacija, u Hrvatskoj je u 2016. godini zabilježeno 1736 osoba koje su se s dijagnozom KBB-a koristile zdravstvenom zaštitom[16]. U razvijenim zemljama više od 70 % uzroka KBB-a čine dijabetička nefropatija, hipertenzivna i aterosklerotska nefroskleroza[13,14]. Postojeći dijagnostički postupak probira KBB-a uključuje najvećim dijelom laboratorijske pretrage kao biomarkere koji su nedovoljno osjetljivi i specifični. Zbog toga se pojavila potreba pronalaženja novih, manje invazivnih, ali istodobno dovoljno osjetljivih i specifičnih biomarkera te inovativnoga terapijskog pristupa. Brojne studije predlažu miRNA kao potencijalne inovativne biomarkere u bolesnika s KBB-om[17,18].

MiRNA visokospecifične za bubreg jesu: **miR-19a**, **miR-19b**, **miR-31**, **miR-146a**, **miR-192**, **miR-194**, **miR-204**, **miR-215**, **miR-216**, **miR-886**. Ekspresija pojedinih miRNA razlikuje se u pojedinim dijelovima bubrega npr. miR-192 izraženije je eksprimirana u korteksu nego u meduli, što je konzistentno s njezinom ulogom u transportu natrija[2,19-21].

Pojedine miRNA koje nisu specifične za bubreg pojačano su eksprimirane i u drugim organima. To su **miR-10a**, **miR-10b**, **miR-21**, **miR-30**, **miR-196a**, **miR-196b**, **miR-451 i miR-let 7a**[21,22].

MikroRNA u bubrežnoj fibrozi

Bubrežna fibroza važno je obilježje gotovo svih KBB-a, a dovodi do završnoga stupnja bubrežne bolesti. Karakterizirana je ili odlaganjem proteina u intersticijski ekstracelularni matriks ili akumulacijom miofibroblasta i destrukcijom bubrežnih tubula. **Transformirajući čimbenik rasta (TGF-β)** jest citokin koji sudjeluje u navedenom procesu bubrežne fibroze[23,24]. TGF-β inducira brojne gene odgovorne za fibrozu poput proteina ektracelularnog matriksa ili mitogenom aktivirane protein-kinaze (MAPK) i regulira pojedine miRNA tijekom bu-

stranded molecules that, like endogenous miRNA, consist of two strands, one of which serves as the guide strand and the other as a passenger strand. The guide strand contains a genetic code identical to the one of the target miRNA molecule. The passenger strand binds to different molecules such as cholesterol, facilitating the insertion of the whole miRNA imitation into individual cells. The fake miRNA is then bonded into the RISC complex, thus creating miRISC which acts like the one created with endogenous miRNA[7,10,12].

Inhibiting overexpressed miRNA can be performed using miRNA sponges or by single-strand antisense oligonucleotides called antimiR. miRNA sponges use transgenetic overexpressed RNA that contains complementary binding locations with repetitions for target miRNA molecules that are to be blocked. As opposed to miRNA, antimiRs are single-strand molecules complementary to the target miRNA molecule, chemically modified to achieve bonding affinity, biostability, and pharmacokinetic characteristics. When they arrive in a cell, antimiRs bind to the target miRNA and inhibit its function[7,12].

MicroRNA in chronic kidney disease

Chronic kidney disease (CKD) is a clinical syndrome characterized by progressive and permanent damage to kidney function – excretion-related, endocrine, and metabolic[13,14]. The incidence of patients with end stage CKD is increasing. In most countries, the estimated prevalence of CKD is 5-14%, increasing up to several times in advanced age[15]. Based on data from Central Health Information System of Croatia and the hospitalization database, 1 736 persons in Croatia were receiving health care for the diagnosis of CKD in 2016[16]. In developed countries, more than 70% of the causes of CKD consist of diabetic nephropathy and hypertensive and atherosclerotic nephrosclerosis[13,14]. The existing diagnostic procedure for CKD differentiation mostly includes laboratory tests for biomarkers that are insufficiently sensitive and specific. Consequently, there was a need for the development of new, less invasive, but also adequately sensitive and specific biomarkers and an innovative treatment approach. Numerous studies have suggested miRNA as potential innovative biomarkers in patients with CKD[17,18].

miRNA that are highly specific to the kidneys are **miR-19a, miR-19b, miR-31, miR-146a, miR-192, miR-194, miR-204, miR-215, miR-216, miR-886**. The expression of individual miRNA differs in individual parts of the kidney; for instance, miR-192 is more expressed in the cortex than in the medulla, which is consistent with its role in sodium transport[2,19-21].

Some miRNA that are not specific to the kidney are more expressed in other organs as well. These are **miR-10a, miR-10b, miR-21, miR-30, miR-196a, miR-196b, miR-451** and **miR-let 7a**[21,22].

MicroRNA in renal fibrosis

Renal fibrosis is an important characteristic of almost all CKD and leads to end stage kidney disease. It is characterized either by deposition of proteins in the interstitial extracellular matrix or myofibroblast accumulation and destruction of the renal tubules. **Transforming growth factor (TGF-β)** is a cytokine that participates in this process of renal fibrosis[23,24]. TGF-β induces numerous genes responsible for fibrosis, such as extracellular matrix proteins or mitogen-activated protein

brežne fibroze. TGF-β1 inducira miR-21, miR-192, miR-491-5p, miR-382, miR-377, miR-214 i miR-433, a suprimira miR-29 i miR-200. Ekspresija većine miRNA promijenjena je u pojedinim bubrežnim bolestima[1,23,24]. Postoji povratna sprega između miRNA i TGF-β signalizacijskog puta.

MikroRNA kao novi biomarker kronične bubrežne bolesti

Istraživanja upućuju na ulogu miRNA kao potencijalnih biomarkera, a ekspresija pojedinih miRNA može se određivati u krvi, serumu, plazmi, mokraći i bubrežnom tkivu dobivenom biopsijom[19].

Brigant *i sur.* istraživali su ulogu miR-126, miR-143, miR-145, miR-155 i miR-223 kao potencijalnih biomarkera u cirkulaciji bolesnika s KBB-om. Bolesnike su podijelili u tri skupine: KBB stadij III. – IV., bolesnici na hemodijalizi i bolesnici s transplatiranim bubregom. Na temelju rezultata miRNA molekule bile su podijeljene u dvije skupine: prvu, miRNA koje su povišene u bolesnika s KBB-om stadija III. – IV. i u bolesnika na hemodijalizi, a snižene u bolesnika s transplantiranim bubregom: miR-143, miR-145 i miR-223. U drugoj skupini su miRNA koje su povišene u bolesnika s KBB-om stadija III. – IV., a snižene u bolesnika na hemodijalizi i u bolesnika s transplatiranim bubregom: miR-126 i miR-155[25].

Szeto *i sur.* istraživali su nalaz miRNA molekula u mokraćnom sedimentu bolesnika s KBB-om. U urinu miRNA mogu biti kvantificirane u sedimentu, ali i u supernatantu koji preostane nakon centrifugiranja. Istraživanje je provedeno na 56 bolesnika s KBB-om u podlozi koje je dijabetička nefropatija, hipertenzivna nefroskleroza i IgA nefropatija. U bolesnika s dijabetičkom nefropatijom pronađena je niska razina **miR-15** u urinu, u bolesnika s hipertenzivnom nefrosklerozom utvrđena je povišena razina **miR-21** i **miR-216a**, dok su bolesnici s IgA nefropatijom imali povišenu razinu **miR-17**[26].

Terapijska uloga mikroRNA u kroničnoj bubrežnoj bolesti

Intenzivno se istražuju u domeni bubrežnih bolesti antimiRNA lijekovi, koji bi potencijalno bili idealnim lijekom u KBB-om: učinkovito blokiraju miRNA u jetri i bubrezima, sigurni su, bez značajnih nuspojava, bolesnici ih mogu samostalno aplicirati (slično inzulinu), imaju dugo vrijeme djelovanja (do 4 tjedna), zbog čega se mogu aplicirati jednom svakih nekoliko tjedana. Osnovni nedostatak antimiRa jest njihova ograničena distribucija u oštećene, cistične i fibrozom promijenjene bubrege[8]. Najbolje je proučen potencijal vezan za **miR-21**. Na životinjskom modelu s reduciranom količinom miR-21 u laboratorijskih se životinja (miš) intersticijska fibroza razvija sporije i u manjoj mjeri u usporedbi s divljim tipom miševa te se na tome temelje terapijski potencijali antimiR-21. Drugi model jest onaj s miševima u kojih je izazvana *null* mutacija alfa3-lanca kolagena tipa 4 (Col4a3-/-) u kojih se razvije Alportov sindrom s KBB-om i zatajenjem bubrega do 11. tjedna. U navedenom je modelu radi usporivanja razvoja i progresije bolesti apliciran antimiR-21 supkutano. Rezultat je bio poboljšanje bubrežne funkcije, usporivanje progresije fibroze, smanjenje albuminurije i sekrecije uremijskih toksina te povećanje medijana preživljenja miševa[7,9,27].

kinase (MAPK), and regulates individual miRNA during renal fibrosis. TGF-β1 induces miR-21, miR-192, miR-491-5p, miR-382, miR-377, miR-214, and miR-433, while suppressing miR-29 and miR-200. Expression of most miRNA is changed in different kidney diseases[1,23,24]. There is a feedback mechanism between miRNA and the TGF-β signalization pathway.

MicroRNA as a new biomarker for chronic kidney diseases

Studies indicate miRNA as a potential biomarker, and the expression of individual miRNA can be determined in the blood, serum, plasma, urine, and kidney tissues extracted though biopsy[19].

Brigant *et al.* studied the role of miR-126, miR-143, miR-145, miR-155, and miR-223 as potential biomarkers in the circulation of patients with CKD. Patients were divided into three groups: CKD stage III-IV, patients on hemodialysis, and patients with transplanted kidneys. Based on the results, miRNA molecules were divided in two groups: the first comprised miRNA that were elevated in patients with CKD stage III-IV and in patients on hemodialysis and were lower in patients with transplanted kidneys: miR-143, miR-145, and miR-223. The second group comprised miRNA elevated in patients with CKD stage III-IV and lower in patients on hemodialysis and patients with transplanted kidneys: miR-126 and miR-155[25].

Szeto *et al.* examined the findings of miRNA molecules in urine sediment of patients with CKD. In urine, miRNA can be quantified in the sediment but also in the supernatant that remains after centrifugation. The study was performed on 56 patients with CKD with underlying diabetic nephropathy, hypertensive nephrosclerosis, and IgA nephropathy. A low level of **miR-15** was found in the urine of patients with diabetic nephropathy, patients with hypertensive nephrosclerosis had an elevated level of **miR-21** and **miR-216a**, whereas patients with IgA nephropathy had elevated levels of **miR-17**[26].

The role of miRNA in the treatment of chronic kidney disease

AntimiRNA drugs are being intensely studied in the domain of kidney diseases, as they could potentially be an ideal medication for CKD: they effectively block miRNA in the liver and kidneys, they are safe with no significant side-effects, patients can self-administer (similarly to insulin), and they have a long action duration (up to 4 weeks), which means that they can be applied once every few weeks. The main drawback of antimiRs is their limited distribution to the damaged, cystic, and fibrous kidneys[8]. The potential of **miR-21** has been studied the most. In an animal model, laboratory animals (mice) with reduced amounts of miR-21 develop interstitial fibrosis more slowly and to a lesser degree in comparison with a wild type of mice, which is what the treatment potential of antimiR-21 is based on. In another model, null mutation of the α3 collagen type 4 chain (Col4a3-/-) was caused in mice, which then developed Alport syndrome with CKD and kidney failure by their 11th week of age. antimiR-21 was subcutaneously administered in this model with the goal or slowing the progression of the disease. The result was improvement in kidney function, reduced progression of fibrosis, reduction in albuminuria and secretion of uremic toxins, and increase in median survival in the mice[7,9,27].

S obzirom na činjenicu da se miRNA nisu znatno evolucijski mijenjale, moguća je brza primjena navedenih antimiRa i u ljudi. AntimiR-21 trenutačno je u fazi 1. kliničkih ispitivanja kao lijek za Alportov sindrom[9,27].

MikroRNA u zatajivanju srca

Zatajivanje srca (HF) kompleksni je sindrom koji može nastati zbog brojnih funkcionalnih ili strukturnih poremećaja srca, a rezultira nemogućnošću srca da održi minutni volumen dostatan da zadovolji metaboličke potrebe organizma. Incidencija HF-a je u porastu te se ubraja u deset vodećih uzroka smrti. Oko 50 % bolesnika s HF-om umire unutar razdoblja od 5 godina. Tri najčešća uzroka HF-a jesu ishemijska bolest srca, dilatativna kardiomiopatija i arterijska hipertenzija. U tijeku su brojna istraživanja koja se bave otkrivanjem novih biomarkera i terapijskih opcija[28]. Brojna su istraživanja upozorila na pojedine miRNA koje bi mogle imati važnu ulogu pa bi stoga u budućnosti miRNA mogle biti inovacija u dijagnostici i liječenju HF-a[6,29].

MikroRNA u fibrozi srca

Fibroza srca podrazumijeva ekscesivnu akumulaciju proteina ektracelularnog matriksa u intersticiju i perivaskularnim regijama te je važan patogenetski čimbenik u razvoju HF-a. Važnu ulogu u fibrozi srca, baš kao i u bubrežnoj, ima TGF-signalni proces, koji aktivira fibroblaste koji se potom diferenciraju u miofibroblaste i luče proteine izvanstaničnog matriksa. Četiri miRNA su utvrđene kao bitni sudionici navedenog procesa. To su: miR-21, miR-29, miR-30 i miR-133. Razina je miR-21 povišena, a razine miR-29, miR-30 i miR-133 snižene[30,31].

MikroRNA kao biomarker zatajivanja srca

„Zlatni standard" biomarkera u HF-u jest NT-proBNP (engl. *N-terminal pro-brain natriuretic peptide*). Brojna su istraživanja proučavala cirkulirajuće miRNA zbog njihove potencijalne uloge kao dijagnostičkih markera u HF-u[6,32]. Osim u dijagnozi bolesti, potencijalne uloge miRNA kao biomarkera bile bi i utvrđivanje etiologije HF-a, razjašnjavanje patofizioloških mehanizama, odabir vrste i intenziteta terapije, kao i praćenje odgovora na terapiju te predviđanje prognoze[6].

Iako se, osim onih u krvi, serumu i plazmi, mogu proučavati i miRNA dobivene biopsijom iz srčanoga tkiva i miRNA iz mononuklearnih stanica dobivenih iz periferne krvi, zasada je najveći interes na cirkulirajućim miRNA[33].

Istraživanja koja su proučavala cirkulirajuće miRNA (miR) u akutnom HF-u dokazala su povišene razine miR-423-5p i miR-499 te snižene razine miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30b, miR-30e-5p, miR-103, miR-106a-5p, miR-142-3p, miR-199a-3p, miR-342-3p i miR-652-3p[6,34-37].

Istraživanja koja govore o promjenama ekspresije pojedinih miR u kroničnom HF-u utvrdila su povišene cirkulacijske razine za: miR-22, miR-92b, miR-122, miR-210, miR-320a, miR-375, miR-423-5p, miR-520d-5p, miR-671-5p, miR-1180, miR-1233 i miR-1908. Sniženu razinu u kroničnom HF-u imale su: miR-30c, miR-107, miR-139, miR-142-5p, miR-146a, miR-183-3p, miR-190a, miR-193b-3p, miR-193b-5p, miR-203, miR-211-5p, miR-221, miR-328, miR-375, miR-494 i miR-558[6,38-43]. Neke od nabrojenih miR mogle bi u budućnosti poslužiti kao biomarkeri akutnog ili kroničnog HF-a.

Given the fact that miRNA have not undergone significant evolutionary changes, there is a chance for swift application of these antimiRs in humans. antimiR-21 is currently in phase 1 clinical trials as a cure for Alport syndrome[9,27].

miRNA in heart failure

Heart failure (HF) represents a complex syndrome that can manifest due to numerous functional or structural disorders of the heart, and results in the inability of the heart to maintain a minute volume adequate for sustaining the metabolic needs of the organism. The incidence of HF is on the rise and has been included among the ten leading causes of death. Approximately 50% of patients with HF die within a period of 5 years. The three most common causes of HF are ischemic heart diseases, dilated cardiomyopathy, and arterial hypertension. There are numerous ongoing studies aimed at discovering new biomarkers and treatment options in HF[28]. Many studies indicated individual miRNA that could have a significant role, which means that miRNA could represent a innovation in the diagnosis and treatment of HR in the future[6,29].

MicroRNA in heart fibrosis

Heart fibrosis refers to the excessive accumulation of extracellular matrix protein in the interstitial and perivascular regions and represents a significant pathogenic factor in the development of HR. A significant role in heart fibrosis, just as in kidney fibrosis, is played by the TGF signalization process, which activates fibroblasts that subsequently differentiate into myofibroblasts and secrete extracellular matrix proteins. Four miRNA have been established as significant participants in this process. These are miR-21, miR-29, miR-30, and miR-133. The levels of miR-21 are elevated, while the levels of miR-29, miR-30, and miR-133 are lowered[30,31].

MicroRNA as a biomarker in heart failure

The gold standard for biomarkers in HF is N-terminal pro-brain natriuretic peptide (NT-proBNP). Numerous studies have examined circulating miRNA for their potential role as diagnostic markers in HF[6,32]. Except in disease diagnosis, the potential roles for miRNA as a biomarker would also be determining the etiology of HF, clarifying the pathophysiological mechanisms, choosing the type and intensity of treatment, prognosis, and monitoring treatment response[6].

Although other than miRNA in blood, serum, and plasma, it is also possible to study miRNA obtained by biopsy of the tissue of the heart as well as miRNA from mononuclear cells obtained from peripheral blood, for now most of the interest has been focused on circulating miRNA[33].

Studies that examined circulating miRNA (miR) in acute heart failure demonstrated elevated levels of miR-423-5p and miR-499 as well as lowered levels of miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30b, miR-30e-5p, miR-103, miR-106a-5p, miR-142-3p, miR-199a-3p, miR-342-3p, and miR-652-3p[6,34-37].

Studies on the changes in the expression of specific miR in chronic HF found elevated circulation levels for: miR-22, miR-92b, miR-122, miR-210, miR-320a, miR-375, miR-423-5p, miR-520d-5p, miR-671-5p, miR-1180, miR-1233, and miR-1908. Lowered levels in chronic HF were found for: miR-30c, miR-107, miR-139, miR-142-5p, miR-146a, miR-183-3p, miR-190a, miR-193b-3p, miR-193b-5p, miR-203, miR-211-5p, miR-221, miR-328, miR-375, miR-494, and miR-558[6,38-43]. Some of the above miR could serve as biomarkers for acute or chronic HF in the future.

Terapijska uloga mikroRNA u zatajivanju srca

Najviše se istražuje potencijalna terapijska uloga miR-1 i miR-133 koje imaju važnu ulogu u hipertrofiji srca te uloga miR-21 i miR-29 za koje je već prije navedeno da sudjeluju u razvoju fibroze srca. Razina je miR-1 u bolesnika s hipertrofijom miokarda snižena, čak i prije pojave kliničkih znakova hipertrofije, što upućuje na njezinu protektivnu ulogu. Animalni model za uporabu miR-1 u štakora s hipertrofijom lijeve klijetke rezultirao je regresijom hipertrofije, smanjenjem fibroze i apoptoze te poboljšanjem signalizacije kalcija[44].

Sličnu ulogu ima i miR-133 u razvoju hipertrofije srca: razina je snižena u bolesnika s hipertrofijom lijeve klijetke, a pojačavanje ekspresije lijekovima baziranih na miRNA pokazalo se kardioprotektivno[45]. Uporabom antimiR-21 u životinjskim modelima (miš) reducirana je aktivnost MAPK-a, što je dovelo da ublaživanja intenziteta fibroze i poboljšanja funkcije srca[46-48].

Za razliku od miR-21, miR-29 ima protektivnu ulogu u razvoju fibroze srca, a njezina snižena ekspresija pogoduje fibrotskim procesima, što, prema Zhang *i sur.*, otvara mogućnost uporabe lijeka temeljenog na miR-29b koji usporuje razvoj fibroze[49].

Zaključak

Kronična bubrežna bolest i zatajivanje srca poprimaju pandemijske razmjere danas u svijetu te su važan globalni javnozdravstveni problem. Epigenetika ima ključnu ulogu u razvoju i fiziologiji, no i u patogenetskim procesima. Dijagnostički je postupak često bez mogućnosti izbjegavanja invazivnih pretraga, a terapija je daleko od mogućnosti zaustavljanja progresije bolesti. Nuždan je razvoj neinvazivnih biomarkera koji bi s visokom osjetljivošću i specifičnošću detektirali bolest te predviđali prognozu i pratili terapijski uspjeh poput miRNA molekula.

Zbog neupitnog porasta incidencije i zdravstvene važnosti KBB-a i HF-a, medicina treba razvijati nove mogućnosti njihove prevencije, dijagnostike i liječenja.

The role of microRNA in the treatment of heart failure

The potential treatment role of miR-1 and mirR-133, which have a significant role in the hypertrophy of the heart, has been studied the most, as well as the role of miR-21 and miR-29, which have already been mentioned above as participating in the development of heart fibrosis. The levels of miR-1 in patients with myocardial hypertrophy is lowered, even before the appearance of clinical signs of hypertrophy, which indicates its protective role. Animal models examining the use of miR-1 in rats with left ventricular hypertrophy have shown that it resulted in regression of hypertrophy, reduction in fibrosis and apoptosis, and improvement in calcium signaling[44].

miR-133 has a similar role in the development of heart hypertrophy: levels are lowered in patients with left ventricular hypertrophy, and strengthening the expression with medication based on miRNA has been shown to be cardioprotective[45]. The use of antimiR-21 in animal models (mice) showed a reduction in MAPK activity, leading to a reduction in the intensity of fibrosis and an improvement of heart function[46-48].

As opposed to miR-21, miR-29 has a protective role in the development of heart fibrosis, and its lowered expression favors fibrotic processes, which opens up the possibility, according to Zhand *et al.*, of the use of medication based on miR-29b that slows the progression of fibrosis[49].

Conclusion

Chronic kidney disease and heart failure are now reaching pandemic proportions at the global level and represent a significant global health care problem. Epigenetics have a key role in development and physiology, but also in pathogenic processes. Diagnostic procedures often cannot avoid invasive tests, and treatment is far from being able to stop the progression of the disease. It is crucial to develop non-invasive biomarker such as miRNA molecules that could detect the disease with high sensitivity and specificity as well as facilitate prognosis and monitor treatment success.

Due to the indubitable increase in the incidence and health care significance of CKD and HF, medical researchers should work on developing new options for their prevention, diagnosis, and treatment.

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