From cellular senescence to age-associated diseases: miRNAs as tools and targets for healthy ageing

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Abstract

miRNAs are the most abundant RNA species to be found in cell-free blood, encapsulated within microvesicles or bound to proteins. miRNAs play essential roles in the regulation of various biological processes. Moreover, specific changes in miRNA transcription levels or miRNA secretory levels have been linked to the development and progression of certain age-related diseases. So, they might be an ideal target for modulating healthy ageing.

Key Words

Ageing, Age-related diseases, miRNA, Senescence.

State of the art

Ageing of the population

The current growth of the world population has major implications for humanity: growing poverty and famine, depletion and pollution of natural resources essential for human survival and migratory pressure from the poor South to the rich North. In absolute terms, the world population has reached 7 billion in 2011. According to the United Nations, it will exceed 8 billion in 2025 and 9 billion by 2045. To be sustainable, the long-term growth rate, i.e. the difference between birth and death rates, should not differ much from 0% [Van Bavel, 2013].

Even though the world population will continue to grow in absolute figures for a certain period, the rate of growth in percentages is decreasing, a result of most countries experiencing a demographic transition from relatively high to low birth and death rates [Canning, 2011]. The decline in mortality and fertility induces the ageing of the population. The percentage of people over 60 in the world has risen from 9.2% in 1990 to 11.7% in 2013 and will be 21% by 2050. It is expected to exceed the number of children for the first time in 2047. Currently about two-thirds of them live in developing countries. They will be concentrated in less developed regions of the world [Christensen et al., 2009].

The ageing of the population has great social and economic consequences. The number of working-age adults per older person is already low in the more developed regions and in some developing countries and should continue to decline in the coming decades with consequent fiscal pressure on support systems for elderly. The increasing mean lifespan of the population is a big success story of humanity, but also poses a challenge that industrialized countries are currently facing, since ageing is associated with increased susceptibility to many diseases like cancer, type 2 diabetes and neurodegenerative disorders [Avery et al., 2014]. So it is necessary to fully understand the mechanisms of ageing in order to prevent its detrimental aspects.

Discussion

Ageing of the cells

A prominent mechanism strongly linked to cellular ageing is cellular senescence, i.e. the arrest of irreversible cellular growth of normal

human cells after serial passages in vitro [Hayflick & Moorhead, 1961]. Critically short telomeres, DNA damage, oncogenic signalling or cellular stress can cause this blockage. Senescent cells differ from other non-dividing cells, such as quiescent ones, by various markers, and morphological changes (however, senescent cells in vivo maintain the normal morphology dictated by tissue architecture). It is interesting to note that senescent cells are characterized by an irreversible growth arrest, altered function / differentiation status, which is reflected by an altered intracellular protein expression and secretion profile, called senescence associated secretory phenotype (SASP). Biomarkers of senescence include absence of proliferative markers, expression of tumor suppressors, cell cycle inhibitors like p21 and/or p16, and often also of DNA damage markers as well as senescence-associated β-galactosidase activity due to GLB1 upregulation [Lee et al., 2006], concomitant with an increase of the lysosomal content of the senescent cells, which allows the lysosomal β-galactosidase to be detected at a suboptimal pH (pH 6.0). This probably reflects the increased autophagy occurring in the senescent cells together with an enlargement of the lysosomal compartment. As previously stated, senescent cells secrete various extracellular factors, including transforming growth factor-β, insulin-like growth factor 1-binding proteins, plasminogen activator inhibitor 1, and inflammatory cytokines and chemokines that can enhance and propagate senescence in autocrine and paracrine mode, as well as tissue remodelling factors. Therefore, senescent cells contribute to the well known pro-inflammatory status of ageing [Campisi & d'Adda di Fagagna, 2007; Muñoz-Espín & Serrano, 2014].

Several studies over the last ten years have clearly shown that senescence has beneficial and harmful effects. In general, transient induction of senescence followed by tissue remodelling is advantageous because it contributes to the elimination of damaged cells. In contrast, persistent senescence or the inability to eliminate senescent cells are harmful. This is particularly relevant in cancer and ageing, both characterized by the accumulation of severe cell damage. Accordingly, senescence is a crucial barrier to cancer progression and senescent cells accumulate with ageing. In summary, senescence is a response selected to eliminate damaged cells. However, with ageing, the complete sequence of senescence-clearance-regeneration is not fully accomplished and senescence may become part of the problem

rather than its solution. Thus, senescence is considered an example of antagonistic pleiotropy and has been classified as an antagonistic sign of ageing [Muñoz-Espín & Serrano, 2014].

On the other hand, an increasing number of studies have been published showing that senescent cells accumulate with age in vivo, contributing to overall ageing and age-related diseases in an organism. The influence of senescent cells in ageing has been demonstrated by reactivating telomerase in mouse tissues, which subsequently became "rejuvenated". Studies in the models clearly show that the elimination of senescent cells delay the ageing process and the onset of age-related diseases. So many scientists are looking for substances that can eliminate senescent cells, that is, senolytic drugs [Jaskelioff et al., 2011; Weilner et al., 2015a,b].

miRNA and ageing

In recent years, the role of miRNAs in ageing has become increasingly evident. They are small non-coding RNA sequences that regulate gene expression through repression of translation. Only recently, however, miRNAs have been found to be secreted in systemic and local environments in which they are protected from RNAses by either carrying proteins or by being packaged into extracellular vesicles (EVs). EVs are vesicles budding from cell membranes (ectosomes), are shedded from multivesicular bodies (exosomes) or derive from apoptotic cells (apoptotic bodies) and contain, depending on their origin, proteins, different RNA species including mRNAs and miRNAs and/or DNAs. Unlike well-known protein-based signalling systems, EVs have the advantage of providing multiple messages, potentially in a synergistic way. The miRNAs are then taken up by recipient cells, modifying the cellular behaviour by the classical miRNA induced silencing of target mRNAs (Figure 1) [Hromada et al., 2017].

The origin of circulating miRNAs, however, is in many cases unclear, although senescent cells emerge as the possible source of such secreted miRNAs. SASP of different types of cells is probably reflected into circulating miRNAs [Weilner et al., 2013]. In vitro, senescent cells secrete more EVs per cell than their quiescent control cells and the amount of secreted vesicles increases over time after induction of stress induced premature senescence. Since differences in circulating miRNAs have been found in a variety of age-related diseases and the accumulation of senescent cells in the elderly emerges as a possible adverse factor in ageing, it is possible to hypothesize

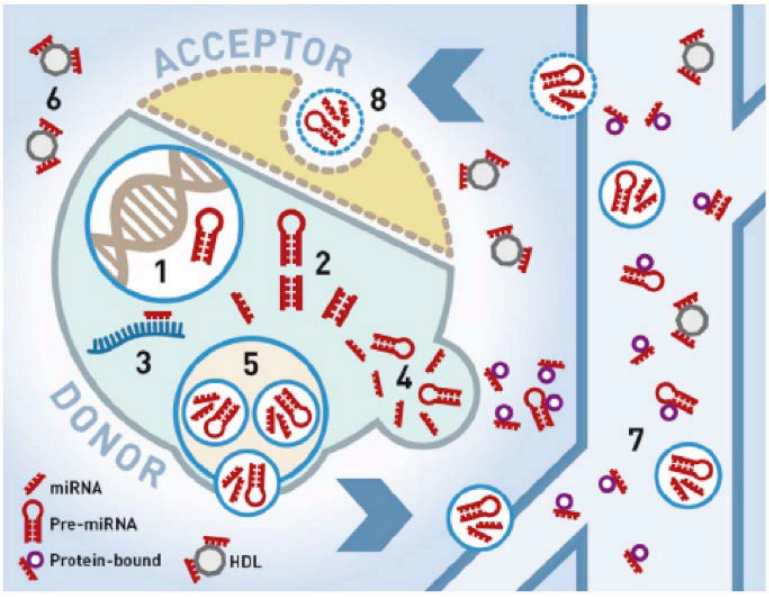

Figure 1. **The concept of circulating microRNAs**. (1) microRNAs are derived from intergenic or intronic genomic regions. In case of intergenic miRNAs, the initial primary transcripts (not shown) are cleaved by Drosha/Dgcr8 to form precursor miRNAs (Pre-miRNA). Alternatively, splicing of intronic miRNAs can give rise to pre-miRNAs. (2) Pre-miRNAs are shuttled via Exportin-5 into the cytoplasm where cleavage by Dicer into double-stranded miRNA duplexes occurs. (3) Single-stranded miRNAs guide the RISC protein complex to target mRNAs resulting in translational repression. (4) mature miRNAs as well as Pre-miRNAs are sorted into ectosomes, which bud from the cell membrane. (5) Intracellularly, multivesicular bodies (MVBs) are formed, which contain exosomes of 50–100 nm size. MVBs fuse with the cell membrane to release miRNA loaded exosomes from the Donor Cell into the supernatant. (6) Lipoprotein or Ago-2 associated miRNAs are present in cell-free liquids. (7) Protein-bound or encapsulated miRNAs are transported with the blood stream. (8) Uptake of extracellular RNA by an Acceptor Cell that is different from the Donor Cell, and potentially located in a different tissue. Reproduced from Hackl M, Heilmeier U, Weilner S, Grillari J. Circulating microRNAs as novel biomarkers for bone diseases - Complex signatures for multifactorial diseases? Mol Cell Endocrinol. 2016; 432: 83-95 under the terms of the Creative Commons Attribution License.

that these miRNAs may contribute to a functional decline observed during ageing [Dellago et al., 2017]. In senescent mesenchymal bone marrow stem cells, the majority of miRNAs are up-regulated, with the exception of miR-199b-5p. This is decreased, thus enhancing the translation of its target, LAMC1, which encodes laminin proteins necessary for cell adhesion and migration as well as signal transduction [Yoo et al., 2014; Hackl et al., 2016].

In an effort to identify miRNAs commonly regulated during ageing, microarray studies were conducted comparing four human replicative cell-ageing models and three organismal ageing models. These diverse model systems shared a set of commonly down-regulated miRNAs, among them members of the miR-17-92 cluster. Several studies using different model systems have confirmed the down-regulation of the miR-17-92 cluster during ageing, and its up-regulation in centenarians considered successful agers [Gombar et al., 2012; Dellago et al., 2017].

On the other hand, in human beings a pilot study analysed 365 circulating miRNA in young, old and centenarians, identifying three general models for circulating miRNAs in young, aged, and long-living individuals. In particular, the miR-21-5p levels were demonstrated to increase in aged, including centenarians. This was replicated in an independent sample group. Based on the expected involvement of miR-21-5p with transforming growth factor-ß signalling and its correlation with other circulating inflammatory molecules, miR-21-5p was proposed as " inflamma-miR", likely linked to the systemic pro-inflammatory status of old people [Olivieri et al., 2012; 2013, Hackl et al., 2016].

In addition to that we recently identified miRNA signatures as biomarkers of an important age-related disease: of osteoporosis. From the notion, that miR-31 is secreted by senescent endothelial cells in vitro, we found that upon EV mediated transfer to mesenchymal stem cells block osteogenic differentiation of these recipient cells [Weilner et al., 2016a]. This prompted us to also test, if other factors from senescent cells might have a synergistic effect and indeed found that Galectin-3, that seems to be involved in modulating Wnt signalling, is pro-osteogenic, but found at low levels in serum as well as in EVs of elderly [Weilner et al., 2016b]. Finally, we set out to identify a signature of miRNAs in serum of osteoporotic fracture patients, which by now is based on more than 700 individually that were analysed using serum based qPCR methods [Heilmer et al., 2016; Kocijan et al., 2016].

Conclusion

Ageing affects different body tissues and is known to have a negative impact on the physiology of cells, tissues and organs, resulting in reduced functionality and regeneration capacity. Since observation in a murine parabiosis models, which, linking the circulation of old animals with young animals, may improve the regenerative potential of old tissue [Conboy et al., 2005], it was clear that circulating factors were important contributors to the ageing phenotype. Consequently, circulating miRNAs have been carefully studied in the context of ageing in recent years [Hackl et al., 2016]. miRNAs are part of the SASP, and are transferred by EVs in a paracrine manner. So, they might be an ideal target for assessing and modulating healthy ageing, especially in the context of osteoporotic fracture risk assessment.

Acknowledgements

The laboratory of J.G. is supported by the BioToP – 'Biomolecular technology of proteins' PhD Programme, Austrian Science Funds (FWF) Project W1224, and the Christian Doppler Society. The financial support by the Austrian Federal Ministry of Economy, Family and Youth, the National Foundation for Research, Technology and Development as well as the FP7 EU projects Frailomic and Sybil are gratefully acknowledged.

Conflict of interest

J.G. is a co-founder of Evercyte GmbH and TAmiRNA GmbH, HD is an employee of TAmiRNA GmbH.

References

Avery P., Barzilai N., Benetos A., Bilianou H., Capri M., Caruso C., Franceschi C., Katsiki N., Mikhailidis D.P., Panotopoulos G., Sikora E., Tzanetakou I.P., Kolovou G. *Ageing, longevity, exceptional longevity and related genetic and non genetics markers: panel statement*. Curr Vasc Pharmacol. 2014; 12: 659-61.

Campisi J., d'Adda di Fagagna F. *Cellular senescence: when bad things happen to good cells*. Nat Rev Mol Cell Biol. 2007; 8: 729–40.

Canning D. *The causes and consequences of demographic transition. Popul Stud Camb*. 2011; 65: 353-61.

Chen J., Patschan S., Goligorsky M.S. *Stress-induced premature senescence of endothelial cells. J Nephrol*. 2008; 21: 337–44.

Christensen K., Doblhammer G., Rau R., Vaupel J.W. *Ageing populations: the challenges ahead*. Lancet. 2009; 374: 1196-208

Conboy I.M., Conboy M.J., Wagers A.J., Girma E.R., Weissman I.L., Rando T.A. *Rejuvenation of aged progenitor cells by exposure to a young systemic environment*. Nature. 2005; 433: 760-4.

Dellago H., Bobbili M.R., Grillari J. *MicroRNA-17-5p: At the Crossroads of Cancer and Aging - A Mini-Review*. Gerontology. 2017; 63: 20-28.

Gombar S., Jung H.J., Dong F., Calder B., Atzmon G., Barzilai N., Tian X.L., Pothof J., Hoeijmakers J.H., Campisi J., Vijg J., Suh Y. *Comprehensive microRNA profiling in B-cells of human centenarians by massively parallel sequencing*. BMC Genomics 2012; 13: 353.

Hackl M., Heilmeier U., Weilner S., Grillari J. *Circulating microRNAs as novel biomarkers for bone diseases - Complex signatures for multifactorial diseases?* Mol Cell Endocrinol. 2016; 432: 83-95.

Hayflick L., Moorhead P.S. *The serial cultivation of human diploid cell strains*. Exp Cell Res. 1961; 25: 585–621.

Heilmeier U., Hackl M., Skalicky S., Weilner S., Schroeder F., Vierlinger K., Patsch J.M., Baum T., Oberbauer E., Lobach I., Burghardt A.J., Schwartz A.V., Grillari J., Link T.M. *Serum miRNA Signatures Are Indicative of Skeletal Fractures in Postmenopausal*

Women With and Without Type 2 Diabetes and Influence Osteogenic and Adipogenic Differentiation of Adipose Tissue-Derived Mesenchymal Stem Cells In Vitro. J Bone Miner Res. 2016; 31: 2173-2192.

Hromada C., Mühleder S., Grillari J., Redl H., Holnthoner W. *Endothelial Extracellular Vesicles-Promises and Challenges*. Front Physiol. 2017; 8: 275.

Jaskelioff M., Muller F.L., Paik J.H., Thomas E., Jiang S., Adams A.C., Sahin E., Kost-Alimova M., Protopopov A., Cadiñanos J., Horner J.W., Maratos-Flier E., Depinho R.A. *Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice*. Nature. 2011; 469: 102–6.

Kocijan R., Muschitz C., Geiger E., Skalicky S., Baierl A., Dormann R., Plachel F., Feichtinger X., Heimel P., Fahrleitner-Pammer A., Grillari J., Redl H., Resch H., Hackl M. *Circulating microRNA Signatures in Patients With Idiopathic and Postmenopausal Osteoporosis and Fragility Fractures*. J Clin Endocrinol Metab. 2016; 101: 4125-4134.

Lee B.Y., Han J.A., Im J.S., Morrone A., Johung K., Goodwin E.C., Kleijer W.J., DiMaio D., Hwang E.S. *Senescence-associated beta-galactosidase is lysosomal beta-galactosidase*. Aging Cell. 2006; 5: 187-95

Muñoz-Espín D., Serrano M. Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol. 2014; 15: 482-96.

Olivieri F., Spazzafumo L., Santini G., Lazzarini R., Albertini M.C., Rippo M.R., Galeazzi R., Abbatecola A.M., Marcheselli F., Monti D., Ostan R., Cevenini E., Antonicelli R., Franceschi C., Procopio A.D. *Age-related differences in the expression of circulating microRNAs: miR-21 as a new circulating marker of inflammaging*. Mech Ageing Dev. 2012; 133: 675-85.

Olivieri F., Rippo M.R., Procopio A.D., Fazioli F. *Circulating inflamma-miRs in aging and age-related diseases*. Front Genet. 2013; 4: 121.

Van Bavel J. *The world population explosion: causes, backgrounds and projections for the future, Facts Views Vis Obgyn*. 2013; 5: 281–291.

Weilner S., Schraml E., Redl H., Grillari-Voglauer R., Grillari J. *Secretion of microvesicular miRNAs in cellular and organismal aging*. Exp Gerontol. 2013; 48: 626-33.

Weilner S., Grillari-Voglauer R., Redl H., Grillari J., Nau T. *The role of microRNAs in cellular senescence and age-related conditions of cartilage and bone: A review*. Acta Orthop. 2015; 86: 92–99.

Weilner S., Skalicky S., Salzer B., Keider V., Wagner M., Hildner F., Gabriel C., Dovjak P., Pietschmann P., Grillari-Voglauer R., Grillari J., Hackl M. *Differentially circulating miRNAs after recent osteoporotic fractures can influence osteogenic differentiation*. Bone. 2015; 79: 43-51.

Weilner S., Schraml E., Wieser M., Messner P., Schneider K., Wassermann K., Micutkova L., Fortschegger K., Maier A.B., Westendorp R., Resch H., Wolbank S., Redl H., Jansen-Dürr P., Pietschmann P., Grillari-Voglauer R., Grillari J. *Secreted microvesicular miR-31 inhibits osteogenic differentiation of mesenchymal stem cells*. Aging Cell. 2016a; 15: 744-54.

Weilner S., Keider V., Winter M., Harreither E., Salzer B., Weiss F., Schraml E., Messner P., Pietschmann P., Hildner F., Gabriel C., Redl H., Grillari-Voglauer R., Grillari J. *Vesicular Galectin-3 levels decrease with donor age and contribute to the reduced osteo-inductive potential of human plasma derived extracellular vesicles*. Aging (Albany NY). 2016b; 8: 16-33.

Yoo J.K., Kim C.H., Jung H.Y., Lee D.R., Kim J.K. *Discovery and characterization of miRNA during cellular senescence in bone marrow-derived human mesenchymal stem cells*. Exp Gerontol. 2014; 58: 139-45..