SHORT TAKE

miR-17, miR-19b, miR-20a, and miR-106a are down-regulated in human aging

Matthias Hackl,1* Stefan Brunner,2* Klaus Fortschegger,1 Carina Schreiner,1 Lucia Micutkova,3 Christoph Mück,3 Gerhard T. Laschober,4 Günter Lepperdinger,4 Natalie Sampson,5 Peter Berger,5 Dietmar Herndl-Biadrstetter,2 Matthias Wieser,1 Harald Kühnel,6 Alois Strasser,6 Mark Rinnerthaler,7 Michael Breitenbach,7 Michael Mildner,8 Leopold Eckhart,8 Erwin Tschachler,8 Andrea Trost,9 Johann W. Bauer,9 Christine Papak,10 Zlatko Trajanoski,10 Marcel Scheideier,10 Regina Grillari-Voglauer,1 Beatrice Grubeck-Loebenstein,2 Pidder Jansen-Dürr3 and Johannes Grillari1

1Aging and Immortalization Research, Department of Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria, Muthgasse 18, A-1190 Vienna
2Departments of Immunology, Molecular and Cell Biology, Extracellular Matrix Research, Endocrinology, Institute for Biomedical Aging Research, Austrian Academy of Sciences, Rennweg 10, 6020 Innsbruck, Austria (IBA)
3Department of Natural Sciences, Institute of Physiology, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Wien, Austria
4Department of Genetics, University of Salzburg, Heilbrunnerstraße 34, 5020 Salzburg, Austria
5Department of Dermatology, Medical University of Vienna, A-1090 Vienna, Austria
6Department of Dermatology, SALK and Paracelsus Medical University, Salzburg, Austria
7Institute for Genomics and Bioinformatics and Christian Doppler Laboratory for Genomics and Bioinformatics, Graz University of Technology, Petersgasse 14, 8010 Graz, Austria

Summary

Aging is a multifactorial process where deterioration of body functions is driven by stochastic damage while continuous stochastic damage contributes to the gradual attenuation of physiological functions, which are partially alleviated by genetically encoded repair systems (Kirkwood, 2008). Genetic determinants, which have a direct impact on these biological processes can be characterized by either studying model organisms that are amenable to genetic manipulations (Lepperdinger et al., 2008), or by applying functional genomic methods. Both strategies have helped to identify genes and proteins, which potentially modulate the aging process. Proteins identified in various studies have been compiled in a database only recently (de Magalhaes et al., 2009). Yet hardly any study has so far addressed the role of miRNAs during aging (Bates et al., 2009; Grillari & Grillari-Voglauer, 2010).

miRNAs are a class of small non-coding silencing RNAs of approximately 22 nucleotides in length (Ghildiyal & Zamore, 2010).
They confer specificity to the RNA-induced silencing complex that either degrades or translationally represses target mRNAs (Bhattacharyya et al., 2006; Carthew & Sontheimer, 2009). As the recognition of target mRNAs mainly depends on the small seed region within the mature miRNA, a single miRNA potentially regulates up to several hundred mRNA targets, thus orchestrating a large variety of cellular processes (Lim et al., 2005; Stefani & Slack, 2008).

Here, we have set out to systematically compare miRNA transcription profiles in old vs. young human cells. Employed were in vitro replicative senescence of endothelial cells (Chang et al., 2005; Hampel et al., 2006), renal proximal tubular epithelial cells (Wieser et al., 2008), skin fibroblasts (Hutter et al., 2002; Stockl et al., 2006) as well as an intra-individual comparison of in vivo replicatively exhausted CD8+ T cells (Saurwein-Teissl et al., 2002; Effros et al., 2005). Furthermore, we used bone-derived mesenchymal stem cells (Fehrer et al., 2007; Laschober et al., 2009), foreskin (Oender et al., 2008), as well as CD8+ CD28+ T cells from old versus young donors (Lazuardi et al., 2009). Detailed characterization of these models as well as an overview over biological and technical replicates and experimental design is presented in the supplements (Figs S1 and S2a).

Locked nucleic acid (LNA)-miRNA microarrays were spotted (Castoldi et al., 2006) using Sanger miRBase v9.2 (Griffiths-Jones et al., 2008) probe sets consisting of 559 human, 170 mouse, and 77 not yet annotated (miRPlus sequences; Exiqon Inc., Vedbaek, Denmark) miRNA probes. Microarray design, a comprehensive set of related protocols as well as raw and normalized intensity data have been submitted to Array Express Database compliant to Minimum Information About a Microarray Experiment standards (Brazma et al., 2001; Brazma, 2009). For Array Express accession numbers and detailed materials and methods see Data S1 and Fig. S2a.

Depending on the experimental system, statistical analysis identified 10–20% of the miRNAs as regulated, while the majority remained unchanged during aging (Table S1). Applying hierarchical clustering of all regulated miRNAs, members of the

![Microarray analysis of differential expression and enrichment of regulated miRNAs in replicative and organismal aging.](image-url)

**Fig. 1** Microarray analysis of differential expression and enrichment of regulated miRNAs in replicative and organismal aging. (a) Size-adjusted Venn diagram depicting the intersection of regulated miRNAs from replicative and organismal aging models: the upper, yellow circle represents five miRNAs that were found significantly regulated (false discovery rate-adjusted \( P \)-value < 0.05) in at least three of the four replicative models. Only miRNAs with uniform up- or down-regulation in all models were considered. For organismal aging experiments, 34 miRNAs were significantly regulated (FDR-adjusted \( P \)-value < 0.05) in at least two of three models, indicated by the lower, purple circle. (b) The intersection contains three miRNAs of the miR-17-92 cluster, namely miR-17, miR-19b, and miR-20a, as well as miR-106a of the paralogous miR-106a-363 cluster. The individual ‘old vs. young’ ratios for these miRNAs, calculated from microarray data, are depicted in a barchart. (c) Fold changes in transcription of young vs. old based on microarrays are given for all members of the miR-17-92 cluster as well as selected miRNAs from paralogous clusters together with 5’ seed sequences. Adjusted \( P \)-values < 0.05 are marked in bold and underlined format, indicating statistically significant regulation.
miR-17-92 cluster and paralogous clusters stood out as being commonly down-regulated (Fig. S3).

Using linear models and moderated T-statistics (Smyth, 2004) in combination with false discovery rate (FDR) adjustment according to Hochberg-Benjamini (1990), we calculated differential expression of miRNAs (FDR-adjusted $P$-value < 0.05) for each of the seven model systems independently (available as tab-delimited sheet in Table S2). Intersections of these lists of regulated miRNAs were then analyzed for the replicative aging models and the ex vivo models, which resulted in five miRNAs with common expression changes in replicative aging, and 34 miRNAs that changed expression during organismal aging (Fig. 1a). Interestingly, both replicative and organismal aging share a common set of four miRNAs ('public miRNAs') that belong to the miR-17-92 cluster or its paralogous cluster miR-106a-363 and are down-regulated in both conditions (Fig. 1b).

Figure 1b shows that microarray analysis identified miR-17 as significantly down-regulated in all seven model systems, while miR-19b and miR-20a are down-regulated in six and miR-106a in five of the seven model systems. Interestingly, three of these four miRNAs also share the same seed sequence (Fig. 1c) indicating a cooperative relief of translational inhibition of a common and important set of target genes. Although low transcription levels of mature miR-17 have been found in S phase cells compared to G0/G1 and G2/M phase HeLa cells (Cloonan et al., 2008), our result is not a mere growth arrest phenomenon, as miR-17 and miR-19b are not regulated in young replicating vs. quiescent endothelial cells (data not shown).

Using quantitative polymerase chain reaction, we confirmed the down-regulation of miR-17, miR-19b, miR-20a, and miR-106a (Fig. 2a) but observed a greater dynamic range in fold changes ranging up to a 6-fold down-regulation, thus indicating an even stronger decrease in the transcription of all four miRNAs with age.

Down-regulation of members of the miR-17-92 cluster has been reported recently in age-related conditions like in stress-induced senescence (Li et al., 2009a), after p53 induction (Brosh et al., 2008) as well as after low level irradiation (Maes et al., 2008a,b) of human fibroblasts.

However, other reports on differential expression of miRNAs in replicative senescence of fibroblasts (Brosh et al., 2008; Lal et al., 2008; Bhaumik et al., 2009; Maes et al., 2009), in senescence of mesenchymal stem cells (Wagner et al., 2008), of human and mouse brain tissue (Lukiw, 2007; Li et al., 2009b) as well as of murine liver (Maes et al., 2008a,b) and lung (Williams et al., 2007; Izzotti et al., 2009) do not explicitly report regulation of miR-17-92 miRNAs. This might either be because of the fact that in some studies only up-regulated miRNAs have been reported or to the strategy that only at least 2-fold changed miRNAs were considered.

As the ability of array platforms to detect fold changes down to 1.3-fold (Wurmbach et al., 2003) and because LNA-based microarray methodology is considered to be one of the most sensitive and reliable at the moment (Willenbrock et al., 2009), we included miR-17-92 cluster members that are significantly regulated even at levels below 2-fold, even more so, because array results also are reported to have the tendency to underestimate the ratios (Wurmbach et al., 2003).

Interestingly, among the published targets of the miR-17-92, cluster is p21/CDKN1A mRNA (Ivanovska et al., 2008; Inomata et al., 2009), we included miR-17-92 cluster members that are significantly regulated even at levels below 2-fold, even more so, because array results also are reported to have the tendency to underestimate the ratios (Wurmbach et al., 2003).
et al., 2009). Indeed, p21/CDKN1A mRNA levels are negatively correlated in all model systems (Fig. 2b), although the ratio in old vs. young T cells does not reach significance.

These data indicate that the miR-17-92 cluster, which is known to contribute to transcriptional regulation in cell cycle control and tumorigenesis (He et al., 2005), also contributes to transcriptional regulation in senescence and aging, consistent with the known interdependence between senescence and tumorigenesis (Campisi, 2003; Rodier et al., 2007).

Among others, E2F transcriptionally activates (Woods et al., 2007) and p53 represses the miR-17-92 cluster (Yan et al., 2009). Thus, decreased miR-17-92 levels are consistent with the notion that, E2F family members decrease (Dimri et al., 1994), while p53 activity increases in senescence (Atadja et al., 1995; Kulju & Lehman, 1995).

Downstream of miR-17-92 are 19 experimentally confirmed mRNA targets besides p21. Many of them are involved in tumorigenesis and cell cycle control (Table S3). Indeed, miR-17-92 suppression induces growth arrest in anaplastic thyroid cancer cell models (Takakura et al., 2008). Furthermore, an increase in the level of miR-17-92 is associated with a decrease in ROS and DNA damage in R8 mutated tumor cells (Ebi et al., 2009). It will be exciting to test in an experimental system whether a decrease in miR-17-92, as detected in our models (Campisi, 2003; Rodier et al., 2007) and p53 represses the miR-17-92 cluster (Yan et al., 2009). Thus, decreased miR-17-92 levels are consistent with the notion that, E2F family members decrease (Dimri et al., 1994), while p53 activity increases in senescence (Atadja et al., 1995; Kulju & Lehman, 1995).

References

Atadja P, Wong H, Garkavtsev I, Veillette C, Riabowol K (1995) Increased activity of p53 in senescing fibroblasts. Proc. Natl. Acad. Sci. U S A 92, 8348–8352.

Bates DJ, Liang R, Li N, Wang E (2009) The impact of noncoding RNA on the biochemical and molecular mechanisms of aging. Biochim. Biophys. Acta 1790, 970–979.

Bhatattacharya SN, Habermacher R, Martine U, Closs EJ, Filipowicz W (2006) Relief of microRNA-mediated translational repression in human cells subjected to stress. Cell 125, 1111–1124.

Bhaumik D, Scott GK, Schokrprur S, Patil CK, Orjalo AV, Rodier F, Lithgow GJ, Campisi J (2009) MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8. Aging 1, 402–411.

Brazma A (2009) Minimum Information About a Microarray Experiment (MIAME) – successes, failures, challenges. Sci. World J. 9, 420–423.

Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Anharse W, Ball CA, Causton HC, Gaasterland T, Glisson P, Holstege FC, Kim IF, Markowitz V, Matese JC, Parkison H, Robinson A, Sarkans U, Schulze-Kremer S, Stewart J, Taylor R, Vilo J, Vingron M (2001) Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat. Genet. 29, 365–371.

Brosig R, Shalgi R, Liran A, Landan G, Korotayev K, Nguyen GH, Enery E, Johnsen H, Buganim Y, Solomon H et al. (2008) p53-Depressed miRNAs are involved with E2F in a feed-forward loop promoting proliferation. Mol. Syst. Biol. 4, 229.

Campisi J (2003) Cancer and ageing: rival demons? Nat. Rev. Cancer 3, 339–349.

Carthew RW, Sontheimer EJ (2009) Origins and Mechanisms of miRNAs and siRNAs. Cell 136, 642–655.

Castoldi M, Schmidt S, Benes V, Noeholm M, Kulozik AE, Hentze MW, Muckenthaler MU (2006) A sensitive array for microRNA expression profiling (miChip) based on locked nucleic acids (LNA). Rna 12, 913–920.

Chang MW, Grillari J, Mayrhofer C, Fortschegger K, Allmaier G, Marzban G, Katinger H, Voglauer R (2005) Comparison of early passage, senescent and hTERT immortalized endothelial cells. Exp. Cell Res. 309, 121–136.

Cloonan N, Brown MK, Steptoe AL, Wani S, Chan WL, Forrest AR, Glenisson P, Holstege FC, Kim IF, Markowitz V, Matese JC, Parkison H, Robinson A, Sarkans U, Schulze-Kremer S, Stewart J, Taylor R, Vilo J, Vingron M (2001) Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat. Genet. 29, 365–371.

Dimri GP, Hara E, Campisi J (1994) Regulation of two E2F-related genes in presenescent and senescent human fibroblasts. J. Biol. Chem. 269, 16180–16186.

Ebi H, Sato T, Sugito N, Hosono Y, Yatabe Y, Matsuyama Y, Yamaguchi T, Osada H, Suzuki M, Takahashi T (2009) Counterbalance between RB inactivation and miR-17-92 overexpression in reactive oxygen species and DNA damage induction in lung cancers. Oncogene 28, 3371–3379.

Effros RB, Dagarag M, Spaulding C, Man J (2005) The role of CD8 T-cell replicative senescence in human aging. Immunol. Rev. 205, 147–157.

Fehrer C, Brunauer R, Laschober G, Unterluggauer H, Reitinger S, Kloss F, Gilly C, Gassner R, Lepperding R (2007) Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. Aging Cell 6, 745–757.

Ghilidyal M, Zamore PD (2009) Small silencing RNAs: an expanding universe. Nat. Rev. Genet 10, 94–108.
Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ (2008) miRBase: tools for microRNA genomics. Nucleic Acids Res. 36, D154–D158.

Grillari J, Grillari-Voglauer R (2010) Novel modulators of aging and longevity: small non-coding RNAs enter the stage. Exp. Gerontol, (in press).

Hampel B, Fortschegger K, Ressler S, Chang MW, Unterluggauer H, Breitwieser A, Sommergruber W, Fitzky B, Lepperding G, Jansen-Durr P et al. (2006) Increased expression of extracellular proteins as a hallmark of human endothelial cell in vitro senescence. Exp. Gerontol. 41, 474–481.

He L, Thomson JM, Hernandez-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM (2005) A microRNA polycistron as a potential human oncogene. Nature 435, 828–833.

Hochberg Y, Benjamini Y (1990) More powerful procedures for multiple significance testing. Stat. Med. 9, 811–818.

Hutter E, Unterluggauer H, Uberall F, Schramek H, Jansen-Durr P (2002) Replicative senescence of human fibroblasts: the role of cell cycle progression in mediating senescence. Exp. Gerontol. 37, 1165–1174.

Inomata M, Tagawa H, Guo YM, Kameoka Y, Takahashi N, Sawada K et al. (2002) Oncogenic role of the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. Mol. Cell. Biol. 28, 2167–2174.

Izzotti A, Calif GE, Steele VE, Croce CM, De Flora S (2009) Relationship of microRNA expression in mouse lung with age and exposure to cigarette smoke and light. FASEB J. 23, 3243–3250.

Jeyapalan JC, Ferreira M, Sedivy JM, Herbig U (2007) Accumulation of senescent cells in mitotic tissue of aging primates. Mech. Ageing Dev. 128, 36–44.

Kirkwood TB (2008) Understanding ageing from an evolutionary perspective. J. Intern. Med. 263, 117–127.

Kulju KS, Lehman JM (1995) Increased p53 protein associated with aging in human diploid fibroblasts. Exp. Cell Res. 217, 336–345.

Lal A, Kim HH, Abdelmohsen K, Kuwano Y, Pullmann R Jr, Srikanth A, Subrahmanyan R, Martindale JL, Yang X, Ahmed F et al. (2008) MicroRNAs in the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. Mol. Cell. Biol. 28, 2167–2174.

Laschober GT, Brunauer R, Jarneg A, Fehrer C, Greiderer B, Lepperding G, Jansen-Durr P et al. (2006) Increased expression of extracellular proteins as a hallmark of human endothelial cell in vitro senescence. Exp. Gerontol. 41, 474–481.

He L, Thomson JM, Hernandez-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM (2005) A microRNA polycistron as a potential human oncogene. Nature 435, 828–833.

Hochberg Y, Benjamini Y (1990) More powerful procedures for multiple significance testing. Stat. Med. 9, 811–818.

Hutter E, Unterluggauer H, Uberall F, Schramek H, Jansen-Durr P (2002) Replicative senescence of human fibroblasts: the role of cell cycle progression in mediating senescence. Exp. Gerontol. 37, 1165–1174.

Inomata M, Tagawa H, Guo YM, Kameoka Y, Takahashi N, Sawada K et al. (2002) Oncogenic role of the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. Mol. Cell. Biol. 28, 2167–2174.

Izzotti A, Calif GE, Steele VE, Croce CM, De Flora S (2009) Relationship of microRNA expression in mouse lung with age and exposure to cigarette smoke and light. FASEB J. 23, 3243–3250.

Jeyapalan JC, Ferreira M, Sedivy JM, Herbig U (2007) Accumulation of senescent cells in mitotic tissue of aging primates. Mech. Ageing Dev. 128, 36–44.

Kirkwood TB (2008) Understanding ageing from an evolutionary perspective. J. Intern. Med. 263, 117–127.

Kulju KS, Lehman JM (1995) Increased p53 protein associated with aging in human diploid fibroblasts. Exp. Cell Res. 217, 336–345.

Lal A, Kim HH, Abdelmohsen K, Kuwano Y, Pullmann R Jr, Srikanth A, Subrahmanyan R, Martindale JL, Yang X, Ahmed F et al. (2008) MicroRNAs in the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. Mol. Cell. Biol. 28, 2167–2174.

Laschober GT, Brunauer R, Jarneg A, Fehrer C, Greiderer B, Lepperding G, Jansen-Durr P (2006) Increased expression of extracellular proteins as a hallmark of human endothelial cell in vitro senescence. Exp. Gerontol. 41, 474–481.

He L, Thomson JM, Hernandez-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM (2005) A microRNA polycistron as a potential human oncogene. Nature 435, 828–833.

Hochberg Y, Benjamini Y (1990) More powerful procedures for multiple significance testing. Stat. Med. 9, 811–818.

Hutter E, Unterluggauer H, Uberall F, Schramek H, Jansen-Durr P (2002) Replicative senescence of human fibroblasts: the role of cell cycle progression in mediating senescence. Exp. Gerontol. 37, 1165–1174.

Inomata M, Tagawa H, Guo YM, Kameoka Y, Takahashi N, Sawada K et al. (2002) Oncogenic role of the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. Mol. Cell. Biol. 28, 2167–2174.

Izzotti A, Calif GE, Steele VE, Croce CM, De Flora S (2009) Relationship of microRNA expression in mouse lung with age and exposure to cigarette smoke and light. FASEB J. 23, 3243–3250.

Jeyapalan JC, Ferreira M, Sedivy JM, Herbig U (2007) Accumulation of senescent cells in mitotic tissue of aging primates. Mech. Ageing Dev. 128, 36–44.
miRNAs in replicative and organismal aging, M. Hackl et al.

Wurmbach E, Yuen T, Sealfon SC (2003) Focused microarray analysis. Methods 31, 306–316.
Yan HL, Xue G, Mei Q, Wang YZ, Ding FX, Liu MF, Lu MH, Tang Y, Yu HY, Sun SH (2009) Repression of the miR-17-92 cluster by p53 has an important function in hypoxia-induced apoptosis. EMBO J. 28, 2719–2732.

Supporting Information
Additional supporting information may be found in the online version of this article:

Fig. S1 Characterization of the analyzed model systems of aging.
Fig. S2 Experimental design of differential miRNA analysis.
Fig. S3 Heatmap visualization and clustering of miRNA expression data.

Table S1 Overview on total numbers of transcribed miRNA as well on miRNAs detected as differentially transcribed.
Table S2 Compilation of all regulated miRNAs in all experimental systems (provided as MS Excel file only).
Table S3 Experimentally validated target mRNAs of the miR-17-92 cluster.
Data S1 Supporting materials and methods.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.