Mechanisms of Ageing and Development 134 (2013) 346–348

Contents lists available at SciVerse ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Short communication

MYCN/LIN28B/Let-7/HMGA2 pathway implicated by meta-analysis of GWAS in suppression of post-natal proliferation thereby potentially contributing to aging

Michael Keane, João Pedro de Magalhães *

Integrative Genomics of Ageing Group, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK

ARTICLE INFO

Article history:
Received 7 December 2012
Received in revised form 12 April 2013
Accepted 19 April 2013
Available online 29 April 2013

Keywords:
HMGA2
LIN28B
Suppression of proliferation
Pace of development
Aging

ABSTRACT

Mammalian organ and body growth slows and finally terminates because of a progressive suppression of cell proliferation, however little is known about the genetic regulatory mechanisms responsible. A meta-analysis of genome-wide association studies using growth and development-related traits revealed that two genes, *HMGA2* and *LIN28B*, had multiple associations. Altered *HMGA2* expression has been shown to result in both overgrowth and pygmy phenotypes in mice and overgrowth in humans. These genes are members of the *MYCN/LIN28B/Let-7/HMGA2* pathway and homologs of *LIN28B* and *let-7* are known to regulate developmental timing in *Caenorhabditis elegans*. Strikingly, expression levels of *let-7* and *Hmga2* in murine stem cells continue to increase and decrease, respectively, after growth terminates, suggesting that this pathway may contribute to regulating the pace of both development and age-related degenerative phenotypes.

Mammalian somatic growth progressively slows postnatally primarily due to a progressive decline in cell proliferation (Chang et al., 2008), however the genetic regulatory mechanisms responsible remain largely obscure (Kennedy and Norman, 2005). Consistent with the antagonistic pleiotropy theory (Williams, 1957), recent results suggest the existence of a multi-organ genetic program suppressing proliferation (PSP) which progressively down-regulates many growth-promoting genes (Lui et al., 2010a) and persists into adulthood, thereby potentially contributing to aging (Lui et al., 2010b).

To identify potential regulatory components of the PSP, a meta-analysis of genome-wide association studies (GWAS) from the National Human Genome Research Institute GWAS catalog (Hindorff et al., 2009) was performed. The 9217 SNPs in the GWAS catalog were filtered for growth and development-related traits resulting in a dataset with 428 SNPs from 45 studies associated with 11 traits. Genes reported in the studies as associated with the SNPs were employed.

Permutation testing is commonly used to determine significance (Johnson et al., 2010) and was employed to estimate the false-discovery rate. In each of 10,000 iterations, all SNPs were randomly and independently assigned to the estimated 22,333 human protein-coding genes retrieved from NCBI Entrez (Pruitt et al., 2009), and the gene with the maximum number of SNPs was identified. Genes with more than two SNPs occurred in 2.43% of the iterations, establishing genes with three or more SNPs as significant. Sixteen reported genes reached this threshold and those with the most associations with multiple developmental traits were *HMGA2* and *LIN28B* with 14 and 7 associated SNPs, respectively (Table 1). In an additional control analysis using 428 randomly selected SNPs, a gene with more than six SNPs was observed in 1.9% of 10,000 iterations, confirming the significance of these two genes.

HMGA2 is a member of the high-mobility group A family that can modulate transcription by altering chromatin structure (Reeves, 2001). Supporting the validity of the association with postnatal proliferation and growth is the case of an individual with a chromosomal inversion truncating this gene, resulting in slightly elevated expression of 1.4 times that of a control (Ligon et al., 2005). Notable phenotypes at 8 years of age were extreme overgrowth in terms of height, weight and head circumference, advanced bone age (~13.5 years) and arthritis. In addition, this individual developed premature dentition, and a panoramic dental X-ray at 4 years indicated advanced dental age. Similarly, expression of a truncated *Hmga2* induced gigantism in transgenic mice (Battista et al., 1999). By contrast, *Hmga2*-null mice demonstrate the "pygmy" phenotype characterized by dramatic reductions in body fat and small stature (Zhou et al., 1995).

LIN28B is a homolog of the *Caenorhabditis elegans lin-28* gene (Guo et al., 2006) which controls developmental timing (Moss et al., 1997). *LIN28B* negatively regulates *let-7* (Piskounova et al., 2011) which in turn is a negative regulator of *HMGA2* (Lee and

* Corresponding author.
E-mail addresses: jp@senescence.info, aging@liverpool.ac.uk (J.P. de Magalhães).

0047-6374/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.mad.2013.04.006

Table 1
The developmental traits used in the meta-analysis and results for the top two genes. The remaining statistically significant genes are: *ADAMTSL3*, *CDK6*, *DLEU7*, *DYM*, *EFEMP1*, *GNA12*, *GPR126*, *HHIP*, *HMGA1*, *LCORL*, *LTBP1*, *MSRB3*, *PLAG1* and *ZBTB38*.

Trait	*HMGA2*			*LIN28B*		
	Number of SNPs	Number of studies	Context(s)	Number of SNPs	Number of studies	Context(s)
Height	8	8	UTR-3 (6) Intergenic Intron	2	2	Intron (2)
Head circumference (infant)	1	1	UTR-3	–		
Brain structure	1	1	Intron	–		
Normalized brain volume	–			–		
Hippocampal volume	–			–		
Primary tooth development (number of teeth)	1	1	Intergenic	–		
Primary tooth development (time to first tooth eruption)	1	1	Intergenic	–		
Permanent tooth development	1	1	Intergenic	–		
Aortic root size	1	1	Intergenic	–		
Menarche (age at onset)	–			4	4	Intron (2) Intergenic (2)
Menarche and menopause (age at onset)	–			1	1	Intron

Dutta, 2007). *Let-7* was the second microRNA discovered and has also been shown to regulate developmental timing in *C. elegans* (Reinhart et al., 2000). Furthermore, *let-7* and the *DAF-12* nuclear hormone receptor engage in reciprocal direct feedback regulation (Hammell et al., 2009), and it was recently shown that upon induction by *DAF-12*, *let-7* can stimulate *DAF-16*/*FOXO* signaling to extend life by targeting *lin-14* and *akt-1* (Shen et al., 2012). This is particularly significant because of the impact of *daf-12* on insulin/IGF-1 signaling (Cypser et al., 2006), which plays a well-established regulatory role in both development and aging (Cohen and Dillin, 2008). In humans, *LIN28B* and *HMGA2* are members of the oncogenic *MYCN*/*LIN28B*/*Let-7*/*HMGA2* pathway (Helland et al., 2011).

It was recently observed that there are dramatic changes in the expression of the *Lin28*/*let-7* axis in the rat hypothalamus during postnatal maturation (Sangiao-Alvarellos et al., 2013), and *LIN28B* over-expression was also shown to increase *MYCN* levels and induce neuroblastoma by suppressing *let-7* (Molenaar et al., 2012). *Mycn* was identified as a transcription factor that is consistently down-regulated during development in multiple mouse and rat organs (Lui et al., 2010a). Similarly, *Hmga2* expression is significantly higher in fetal than young–adult stem cells (Kiel et al., 2005), and it is required to maintain stem cell self-renewal in multiple tissues (Nishino et al., 2008). Furthermore, *Hmga2* levels inversely correlate with expression of *let-7* (Mayr et al., 2007). While it is possible that additional mechanisms influence phenotypes, perhaps via early life effects, *Hmga2* was not found to be required for stem cell formation during embryonic development (Nishino et al., 2008).

These findings indicate that the *MYCN*/*LIN28B*/*Let-7*/*HMGA2* pathway may be an important regulatory component of the PSP.

Because body size is maintained following growth termination, it might be expected that *let-7* and *Hmga2* levels in stem cells would stabilize. Therefore it is particularly notable that, to the contrary, they continue to increase and decrease in expression, respectively, coinciding with increasing expression of $p16^{Ink4a}$, a potent tumor suppressor (Nishino et al., 2008). $p16^{Ink4a}$ and $p19^{Arf}$ levels in stem cells are negatively regulated by *Hmga2* (Nishino et al., 2008) and over-expression of $p16^{Ink4a}$ with age has been reported to decrease stem cell self-renewal in mice (Molofsky et al., 2006). This suggests that the PSP continues to progress into adulthood, which has been hypothesized to contribute to aging (Lui et al., 2010b). Further supporting this hypothesis is gene expression data showing that many of the changes that occur during aging originate during development and that cell-cycle-related genes are strongly over-represented among genes that persistently decline in expression throughout postnatal life (Lui et al., 2010b). In addition, increasing expression of *let-7* has been shown to contribute to declining germ-line stem cell self-renewal in *Drosophila* (Toledano et al., 2012) and human neurodegeneration (Lehmann et al., 2012). A QTL encompassing *Hmga2* has also been associated with longevity in mice (Klebanov et al., 2001).

Because continuation of the PSP after growth terminates will ultimately cause deleterious degenerative phenotypes, it could be assumed that it would have been strongly selected against. One possibility is that it escaped further selective pressure once manifestation of these phenotypes was delayed until the end of the typical reproductive lifespan (de Magalhães, 2012), during which it might also have a fitness-enhancing effect by slightly reducing cancer risk and energy requirement. It could also be expected that a steady decrease in body size would be observed due simply to net cell loss, which clearly conflicts with reality. Conversely it has been demonstrated that senescent cells accumulate in mammalian tissue from early adulthood (Herbig et al., 2006). However not enough is currently known to support firm conclusions about mechanisms maintaining organ and body size.

Taken together, these results link this pathway to a growth-regulation process potentially relevant to aging, hence it merits further studies. *Hmga2*-null mice have been proposed as a model to test if cell divisions contribute to aging (de Magalhães and Faragher, 2008). While their increased suppression of stem cell proliferation could in isolation be expected to decrease lifespan, a probable confounding factor is their small body size which likely results in reduced demand on stem cell pools. Indeed, these opposing effects suggest a possible explanation for the puzzling observation that the correlation of longevity with body size is negative intra-species but positive inter-species (Miller et al., 2002). In larger species such as humans relative to mice, the greatly increased chronological delay in the induction of $p16^{Ink4a}$ (Kim and Sharpless, 2006) suggests that the rate of change in expression of its regulators *HMGA2* and *let-7* has correspondingly been significantly reduced, with a positive effect on longevity. However if this discount rate of stem cell self-renewal is intra-specifically consistent, it appears plausible that smaller individuals would experience a slower rate of tissue degeneration due simply to a lower total cellularity representing a reduced cell replacement burden on stem cell pools. Therefore while the net impact on the lifespan of *Hmga2*-null mice is difficult to predict, it seems improbable that no longevity effects would be observed. The timing of growth termination in these animals relative to wild type may indicate which effect is dominant. An alternative experiment would be to maintain expression of one or more members of this

pathway at growth termination levels, perhaps using transgenic *Drosophila* and in particular the known orthologs *lin-28* and *let-7*. It could reasonably be anticipated that a modest increase in energy requirement and hyperplasia together with a significant attenuation of age-related degenerative phenotypes would be observed.

Acknowledgements

The authors would like to thank Daniel Wuttke for critical reading of the manuscript. MK is supported by a studentship from the University of Liverpool's Faculty of Health and Life Sciences.

References

Battista, S., Fidanza, V., Fedele, M., Klein-Szanto, J.P., Outwater, E., Brunner, H., Santoro, M., Croce, C.M., Fusco, A., 1999. The expression of a truncated HMGIC gene induces gigantism associated with lipomatosis. Cancer Research 59, 4793–4797.

Chang, M., Parker, E.A., Muller, T.J., Haenen, C., Mistry, M., Finkielstain, G.P., Murphy-Ryan, M., Barnes, K.M., Sundaram, R., Baron, J., 2008. Changes in cell-cycle kinetics responsible for limiting somatic growth in mice. Pediatric Research 64, 240–245.

Cohen, E., Dillin, A., 2008. The insulin paradox: aging, proteotoxicity and neurodegeneration. Nature Reviews. Neuroscience 9, 759–767.

Cypser, J.R., Tedesco, P., Johnson, T.E., 2006. Hormesis and aging in *Caenorhabditis elegans*. Experimental Gerontology 41, 935–939.

de Magalhães, J.P., 2012. Programmatic features of aging originating in development: aging mechanisms beyond molecular damage? FASEB Journal 26, 4821–4826.

de Magalhães, J.P., Faragher, R.G.A., 2008. Cell divisions and mammalian aging: integrative biology insights from genes that regulate longevity. BioEssays 30, 567–578.

Guo, Y., Chen, Y., Ito, H., Watanabe, A., Ge, X., Kodama, T., Aburatani, H., 2006. Identification and characterization of lin-28 homolog B (LIN28B) in human hepatocellular carcinoma. Gene 384, 51–61.

Hammell, C.M., Karp, X., Ambros, V., 2009. A feedback circuit involving let-7-family miRNAs and DAF-12 integrates environmental signals and developmental timing in *Caenorhabditis elegans*. Proceedings of the National Academy of Sciences of the United States of America 106, 18668–18673.

Helland, Å., Anglesio, M.S., George, J., Cowin, P.A., Johnstone, C.N., House, C.M., et al., 2011. Deregulation of MYCN, LIN28B and LET7 in a molecular subtype of aggressive high-grade serous ovarian cancers. PLoS ONE 6, e18064.

Herbig, U., Ferreira, M., Condel, L., Carey, D., Sedivy, J.M., 2006. Cellular senescence in aging primates. Science 311, 1257.

Hindorff, L.A., Sethupathy, P., Junkins, H.A., Ramos, E.M., Mehta, J.P., Collins, F.S., Manolio, T.A., 2009. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proceedings of the National Academy of Sciences of the United States of America 106, 9362–9367.

Johnson, R.C., Nelson, G.W., Troyer, J.L., Lautenberger, J.A., Kessing, B.D., Winkler, C.A., O'Brien, S.J., 2010. Accounting for multiple comparisons in a genome-wide association study (GWAS). BMC Genomics 11, 724.

Kennedy, D., Norman, C., 2005. What don't we know? Science 309, 75.

Kiel, M.J., Iwashita, T., Yilmaz, O.H., Morrison, S.J., 2005. Spatial differences in hematopoiesis but not in stem cells indicate a lack of regional patterning in definitive hematopoietic stem cells. Developmental Biology 283, 29–39.

Kim, W.Y., Sharpless, N.E., 2006. The regulation of INK4/ARF in cancer and aging. Cell 127, 265–275.

Klebanov, S., Astle, C.M., Roderick, T.H., Flurkey, K., Archer, J.R., et al., 2001. Maximum life spans in mice are extended by wild strain alleles. Experimental Biology and Medicine (Maywood, NJ) 226, 854–859.

Lee, Y.S., Dutta, A., 2007. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. Genes & Development 21, 1025–1030.

Lehmann, S.M., Kruger, C., Park, B., Derkow, K., Rosenberger, K., Baumgart, J., et al., 2012. An unconventional role for miRNA: let-7 activates Toll-like receptor 7 and causes neurodegeneration. Nature Neuroscience 15, 827–835.

Ligon, A.H., Moore, S.D.P., Parisi, M.A., Mealiffe, M.E., Harris, D.J., Ferguson, H.L., Quade, B.J., Morton, C.C., 2005. Constitutional rearrangement of the architectural factor HMGA2: a novel human phenotype including overgrowth and lipomas. American Journal of Human Genetics 76, 340–348.

Lui, J.C., Forcinito, P., Chang, M., Chen, W., Barnes, K.M., Baron, J., 2010a. Coordinated postnatal down-regulation of multiple growth-promoting genes: evidence for a genetic program limiting organ growth. FASEB Journal 24, 3083–3092.

Lui, J.C., Chen, W., Barnes, K.M., Baron, J., 2010b. Changes in gene expression associated with aging commonly originate during juvenile growth. Mechanisms of Ageing and Development 131, 641–649.

Mayr, C., Hemann, M.T., Bartel, D., 2007. Disrupting the pairing between let-7 and HMGA2 enhances oncogenic transformation. Science 315, 1576–1579.

Miller, R.A., Harper, J.M., Galecki, A., Burke, D.T., 2002. Big mice die young: early life body weight predicts longevity in genetically heterogeneous mice. Aging Cell 1 (1) 22–29.

Molenaar, J.J., Domingo-Fernández, R., Ebus, M.E., Lindner, S., Koster, J., et al., 2012. LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression. Nature Genetics 44, 1199–1206.

Molofsky, A.V., Slutsky, S.G., Joseph, N.M., He, S., Pardal, R., Krishnamurthy, J., Sharpless, N.E., Morrison, S.J., 2006. Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. Nature 443, 448–452.

Moss, E.G., Lee, R.C., Ambros, V., 1997. The cold shock domain protein LIN-28 controls developmental timing in *C. elegans* and is regulated by the lin-4 RNA. Cell 88, 637–646.

Nishino, J., Kim, I., Chada, K., Morrison, S.J., 2008. Hmga2 promotes neural stem cell self-renewal in young but not old mice by reducing p16Ink4a and p19Arf expression. Cell 135, 227–239.

Piskounova, E., Polytarchou, C., Thornton, J.E., Lapierre, R.J., Pothoulakis, C., Hagan, J.P., Iliopoulos, D., Gregory, R.I., 2011. Lin28A and Lin28B inhibit let-7 microRNA biogenesis by distinct mechanisms. Cell 147, 1066–1079.

Pruitt, K.D., Tatusova, T., Klimke, W., Maglott, D.R., 2009. NCBI reference sequences: current status, policy and new initiatives. Nucleic Acids Research 37 (Database) D32–D36.

Reeves, R., 2001. Molecular biology of HMGA proteins: hubs of nuclear function. Gene 277, 63–81.

Reinhart, B.J., Slack, F.J., Basson, M., Pasquinelli, A.E., Bettinger, J.C., Rougvie, A.E., Horvitz, H.R., Ruvkun, G., 2000. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 403, 901–906.

Sangiao-Alvarellos, S., Manfredi-Lozano, M., Ruiz-Pino, F., Navarro, V.M., Sánchez-Garrido, M.A., Leon, S., Dieguez, C., Cordido, F., Matagne, V., Dissen, G.A., Ojeda, S.R., Pinilla, L., Tena-Sempere, M., 2013. Changes in hypothalamic expression of the Lin28/let-7 system and related microRNAs during postnatal maturation and after experimental manipulations of puberty. Endocrinology 154 (2) 942–955.

Shen, Y., Wollam, J., Magner, D., Karalay, O., Antebi, A., 2012. A steroid receptor-microRNA switch regulates life span in response to signals from the gonad. Science 338, 1472–1476.

Toledano, H., D'Alterio, C., Czech, B., Levine, E., Jones, D.L., 2012. The let-7-Imp axis regulates ageing of the *Drosophila* testis stem-cell niche. Nature 485, 605–610.

Williams, G.C., 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11, 398–411.

Zhou, X., Benson, K.F., Ashar, H.R., Chada, K., 1995. Mutation responsible for the mouse pygmy phenotype in the developmentally regulated factor HMGI-C. Nature 377, 771–774.