Increased Expression, but Not Insertion of L1 Retrotransposons in Aged Neural Stem and Progenitor Cells

Jonas Fritze[1] and Henrik Ahlenius[2]

Stem Cell Aging and Neurodegeneration group, Lund Stem Cell Center. Lund University, Sweden. [1]Author. [2]Supervisor.

Keywords: Retrotransposons, Aging, Neural Stem Cells, LINE1, APOBEC3

Retrotransposons are mobile elements that build up an extensive part of our genome, and can create genomic instability through new insertions. LINE1 is a subtype of transposons that are still active in human and mice, and can both copy themselves, and assist other retroelements to new insertions, but are strongly regulated by different enzymes. Neural stem and progenitor cells (NSPCs) are present in the developing and adult brain and ensure neurogenesis throughout life but declines with advancing age. So far, very little is known about retrotransposon activity during aging and nothing about their expression or function in aging NSPCs. Here we have compared retrotransposon expression and integration in NSPCs from adult and aged subventricular zone in mice. We show that L1 retroevents take place in adult NSPCs, and that expression of L1 and its regulator APOBEC3 are substantially increased in aged compared to adult NSPCs but without any evidence for accumulation of new genomic insertions.

Introduction

Adult neurogenesis. Neural stem cells (NSCs) are multipotent cells within the developing and adult central nervous system that have extensive self-renewal and proliferation capacity. In contrast, their immediate progeny vary in potency and eventually terminally differentiate to neurons, astrocytes or oligodendrocytes [1-3]. It is in practice difficult to separate bonafide NSCs and progenitors from each other due to lack of efficient markers [4-9], and together they are therefore commonly referred to as neural stem/progenitor cells (NSPCs) even though NSCs has been identified as the slower dividing subtype of the pool [10]. Neurons are produced from NSPCs throughout life in the subventricular zone (SVZ) and the subgranular zone (SGZ) of the adult mammalian brain through a process termed neurogenesis, to maintain homeostasis and potentially replace damaged tissue [11-15]. The SVZ lies adjacent to the lateral ventricles and is directly connected to the cerebrospinal fluid (CSF) [16] in resemblance to most NSCs during early development [17], but different from the SGZ, a region located in the dentrate gurys (DG) and part of the hippocampus. In rodents, NSCs produce transient amplifying progenitors which then gives rise to neuronal restricted progenitors called neuroblasts that migrate from the SVZ via the rostral migratory stream (RMS) to the olfactory bulb (OB) where they differentiate into interneurons [18-20]. Neurogenesis in the OB is essential for olfaction and olfactory memory [21-23]. Similar to the SVZ, NSCs in the SGZ also gives rise to transit amplifying progenitors and neuroblasts, but in this region neuroblasts only migrate a short distance to give rise to dentate granular neurons [24]. Neurogenesis in the hippocampus is believed to be important for memory and learning [25, 26].

Aging in the neuronal stem cell niches. During aging, a process associated with decline in ability to maintain homeostasis and tissue repair [27, 28], NSPCs play a unique role by passing on part of their phenotype to newly generated neurons [29, 30] and with requirements to withstand both chronological (quiescent NSCs) and long-term replicative aging in order to ensure continuous neurogenesis [31, 32]. The most common theory explaining aging [33] is macro molecular decline such as stochastic DNA damage [34]. Indeed, higher accumulation of mutations [35] and increased senescence [36] have been shown to decrease the pool of NSPCs in aged animals [37, 38], this together with a declined expression of genes for neurogenic stem cell markers, transcription factors [38], and growth factors have been reported to reduce neurogenesis in both stem cell niches [39, 40]. Furthermore, neurogenesis in the SVZ has also been demonstrated to decrease with advancing age due to telomere shortening [41, 42] deterioration of ventral SVZ ependyma adjacent to the CSF [37, 43] and reduced growth factor signaling [44, 45], all leading a decline in neuroblast formation and proliferation [38, 44, 45]. However, aged SVZ NSPCs have been shown to retain similar capacity for differentiation and to form functional neurons when isolated in vitro, albeit with reduced efficiency [38]. Similar results have been equivalently indicated in the SGZ [46, 47]. Deficient olfactory discrimination in old mice [44] suggests connections between diminished neurogenesis and the impairment of neural networks involved in olfaction [48].

Degree project in Applied Biochemistry for Engineers (LTH). Published March 24, 2015
Author: Jonas Fritze. Email: Jonas.Fritze@med.lu.se
Supervisor: Henrik Ahlenius (LU) Lund Stem Cell Center, Stem Cell Aging and Neurodegeneration group
Examinator: Leif Bülow (LTH) Pure and Applied Biochemistry, Gene group

Transposable elements. A large portion of our genome originate from mobile DNA elements [49] capable of incorporating or copying themselves into new, seemingly random, positions of the genome [50-53] with possibility to result in advantageous mutations for the host [54-57], but more often cause instability [58-60]. Most elements are dormant, but still active in human and mice are the long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) [61, 62] which are part of the retrotransposon class that use an RNA intermediate to mobilize, and the subclass of non-long terminal repeats (non-LTR) [63, 64]. LINE1 (L1) is the only autonomous subtype in humans [65, 66], with a machinery that have been co-opt by other transposons to mobilize [67, 68]. However, Intracisternal A-particle elements (IAPs), part of the LTR class, are also active and autonomous in mice [69, 70]. L1 includes a 5' untranslated region (5'UTR) where a DNA polymeras II (Pol II) promotor sequence is located [71], the open reading frame 1 (ORF1) that encodes an RNA-binding protein [72-74], the open reading frame 2 (ORF2) that express an endonuclease [75], a reverse transcriptase [76, 77] and a 3'-UTR terminal followed by a poly(A) tail [71]. The L1 cycle starts in the nucleus where transcription takes place, mRNA is then exported to the cytoplasm where the protein complex is translated before reentering the nucleus for reversed transcription and insertion (reviewed in[78]). Although few L1 are active in humans [79, 80], it has been suggested that Alu elements (human SINEs) can mobilize despite inactive L1 loci [81].

L1 regulators. Since retrotransposons can be harmful to the genome, at least when development have ended, multiple mechanisms to control their activation and integration have evolved. L1 is down-regulated by apolipoprotein B mRNA editing enzyme catalytic polypeptide 3 (APOBEC3) [82, 83] through cytidine deamination during reverse transcription [84-86], similar to Activation-induced cytidine deaminase (AID) [87] that also has been shown to restrict L1 through cytidine deamination [88]. Methyl-CpG-binding protein 2 (MECP2) [89, 90] can inhibit transcription while binding to chromatin and methylated DNA at the L1 promotor [91-94], and KRAB-associated protein 1 (KAP1), also known as TRIM28 or TIF1β, repress endogenous retroviruses (ERVs) in NSPCs [95-97] by attaching to ERVs via sequence specific Kruppel-assosiated box zinc-finger proteins (KRAB-ZFPs) [98, 99], and inducing histone modifications by recruiting multiprotein complexes [100, 101] that opens up for DNA methyltransferases and cytosine methylation [102-104]. Other mechanism suggested to influence L1 activity, are small interfering RNAs (siRNA) [105-108], the Trex1 DNA exonuclease [109] and ataxia telangiectasia mutated (ATM) [110]. The L1 promotor contains binding sites for SOX2 among other transcription factors that are involved in neurogenesis [111-114], and is down-regulated by SOX2 [89, 90]. As NSPCs start to differentiate, SOX2 and MECP2 decrease, leading to a boost in L1 activity during early divisions [89, 90, 115]. L1 activity also increase with age in human dermal fibroblasts as interaction between KAP1 and heterochromatin lessens [116]. Interestingly somatic retrotransposition occur more frequently in neurons then in other neural cell types [89, 115, 117], and are accumulated in aged neurons [118].

Retrotransposons in disease and aging. Retroelements has been connected to several human diseases [119, 120], for instance neurodegenerative disorders such as Ataxia [121] and Pyruvate dehydrogenase complex deficiency (PDCD) caused by L1 [122], as well as increased L1 mobilization in schizophrenia [117]. Transpositioning has been linked with genomic imbalance during chronological aging in yeast [123], and de novo L1 insertions in human lung tumors suggest that retroelement induced genomic instability have a major impact on cancer development [124], although not occurring as frequently in the brain [124, 125]. Indications of increased neuronal genomic variation with advancing age [126, 127] strengthen the possible relationship between transposons ability to cause DNA damage [128-130] and aging [34, 131], which is known to exponentially increase the risk for developing degenerative brain disorders [132, 133]. It has been shown that L1 are active and mobilize in human neural precursor cells (NPCs) [115] as well as rat- and mouse hippocampal NSPCs [89, 134], but it is still unexplored if transposable elements have a correlation with the aging process of NSPCs. An increased activity of retrotransposons leading to genomic instability of NSPCs could very well interfere with and in part explain the reduced neurogenesis seen in aging animals. Here we have investigated the possible changes in activity of LINE1, SINE and IAP retrotransposons, as well as their regulators KAP1, APOBEC and MECP2 between adult and aged mice. We show for the first time that expression of LINE1 is increased in aged NSPCs and that this process is accompanied by a compensatory increase in APOBEC3.

Materials and methods

Animals. All experimental procedures were approved by Malmö-Lund Ethical Committee and were in accordance with European Union directive on the subject of animal rights. Five adult (3 months), and five aged (20 months) C57bl6 mice were purchased from Janvier labs and housed in the animal facility connected to Lund Univeristy Biomedical Center at 22°C, 40-60% humidity, and a 12hr light/dark cycle with ab libitium access to food and water.

Dissection. Brains were collected as described [135]. Briefly, animals were sacrificed by cervical dislocation and sprayed with 70% ethanol after which brains were removed and put in ice-cold L-15 medium (Invitrogen). The brains were then sectioned into 1 mm thick coronal slices using a brain matrix and microtome blades. After sectioning the hippocampus (HC), cerebral cortex (CTX) and olfactory bulb (OB) were dissected, placed in 700µl RLT lysis buffer (Qiagen) supplemented with 1% 2-mercaptoethanol (Sigma) and stored at -80°C. Subsequently, the subventricular zone (SVZ) was dissected, put temporarily in ice cold L-15 medium, and later dissociated in 5ml papain, dispase and Dnase1 (PPD) solution (Sigma) at 37°C for 30 min. Cells were then pelleted, resuspended and purified through a two-step gradient, starting with 10min centrifugation at 2000rpm in 2ml Hanks balanced salt solution

(HBSS)-sucrose (0.3g/ml sucrose in 0.5x HBSS (Invitrogen)) followed by a 7min centrifugation at 1500rpm in 200ul Earls balanced salt solution (EBSS) (Invitrogen) overlayed on top of 1ml bovine serum albumin (BSA) and HEPES containing EBSS (0.04g/ml BSA (Sigma) and 2% HEPES in EBSS). The final pellet was then used for cell culture.

Cell culture. All cell lines were grown in a humidified incubator set to 5% CO2, and 37°C. NSPCs from adult and aged SVZ were expanded in parallel, as described [135], in DMEM/F12 + L-glutamine/Glutamax™ (Gibco) supplemented with 2% B27 (Gibco), 1% penicillin streptomycin (Sigma), 10 ng/ml EGF and 20 ng/ml bFGF, passaged when spheres reached 200-300µm in diameter (~1week) with Accutase (Gibco), fed every second day with growth factors and added additional fresh medium four days after passage (50% of total volume. When expanded as monolayers, culture surfaces were coated with Laminin (Sigma) over Poly-L-Lysine (PLL, Sigma), and Euromed-N (EuroClone) with B27, 1% L-glutamine, EGF and FGF (Gibco) was used as culture medium. ~70% of the medium was changed every second day together with added growth factors (calculated for the total volume), and passaged after reaching ~80% confluency with Trypsin-EDTA (Gibco). HEK 293t cells were grown as monolayers on PLL coated surfaces in DMEM/F12 Glutamax (4.5g/l D-glucose) (Gibco) supplemented with 10% FBS (Gibco), 1% sodium pyrovate, 1% NEAA and 1% Pen Strep. ~70% of the medium was changed every 2-3 days.

Transfection. ~$4 \cdot 10^6$ cells from dissociated neurospheres during passage 8 and 9 were transfected for each condition by electroporation with Nucleofector I, program A-033 (Amaxa), using Mouse NSC Nucleofector Kit (Amaxa) according to manufacturer's guidelines. HEK 293t cells were transfected using Fugene (Promega) following manufacturer's protocol, with 0.5µl plasmid DNA and 7/3µl Fugene in DMEM/F12 + L-glutamine/Glutamax™ (Gibco) to a total volume of 25µl per well (9.4cm^2 growth area) when cells reached ~70% confluency.

Plasmids. EF06R [136], provided by Eline Luning Prak (Addgene plasmid # 42940), containing the $L1_{RP}$ element tagged with an enhanced green fluorescent protein (EGFP) cassette ($pL1_{RP}$-EGFP) was used during transfection to investigate the occurrence of retrotransposition. EF05J [136], provided by Eline Luning Prak (Addgene plasmid # 42941), similar to EF06R but with disabling mutations in ORF1 served as negative control. PmaxGFP (Lonza) was acting positive control during Nucleofection, and FUW-TetO-EGFP when using Fugene.

Immunohistochemistry. Transfected cells grown as monolayers were fixed for 25min at RT with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS), washed 3x with 0.1 M potassium-PBS (KPBS) and once with pre-incubation solution consisting of 0.025% Triton X-100 and 5% normal donkey serum (NDS) in KPBS, before primary antibody incubation were carried out overnight at 4°C in pre-incubation solution. Primary antibodies included mouse anti Nestin (1:100, MAB353, Millipore) and rabbit anti GFP (1:1000, AB290-50, Abcam). Cells were then rinsed 2x with 0.025% Triton X-100 in KPBS and once with pre-incubation solution, followed by secondary antibody incubation including 0.1% Hoechst (Sigma), 0.025% Cy3 conjugated donkey anti mouse (1:500, Jackson ImmunoResearch) and 488 conjugated donkey anti rabbit (1:500, Jackson ImmunoResearch) for 2h in pre-incubation solution at room temperature in the dark. Finally cells were washed once with 0.025% Triton X-100 in KPBS, twice with KPBS, and left in KPBS for analysis.

Microscopial analysis. Using the inverted fluorescent microscope IX51 (Olympus) all immunostained cells were methodically examined with 20x magnification for GFP, and then analyzed for co-expression of nestin and nuclear integrity by hoechst. Pictures were taken through the culture vessel using a DP21 camera (Olympus) coupled to the microscope.

RNA & DNA isolation. Total RNA and DNA was isolated from CTX, OB, HC and cells expanded from the SVZ at passage 2-3 and 9-10 using DNA/RNA/Protein mini kit (Qiagen) according to manufacturer's instructions. Breifly, samples, collected and frozen at -80 in RLT lysis buffer, was brought close to RT and homogenized by pipetting and passed through QIA-shredders. On column DNase digestion (Qiagen) was performed during RNA purification, and the final elution of RNA was carried out twice with 30µl H_2O to a final volume of 60µl, DNA two times with 50µl H_2O to a total volume of 100µ. Concentrations were measured on a Nanodrop 2000 spectrophotometer (ThermoFisher Scientific).

Quantitative RT-PCR. For cDNA synthesis, 300ng RNA was used together with the qScript cDNA Synthesis Kit (Quanta) in a Thermal cycler PTC-200 (Biorad) following manufacturer's guidlines. 96-well MicroAmp plates (Applied Biosystems) was prepared with Power SYBR Green (life technologies), 0.625pmol of forward and reverse primers (*table1*) and 10ng cDNA, or 5ng genomic DNA, in a total volume of 10µl per well. RT-qPCR was performed in the i-Cycler (Bio-Rad) connected to a IQ5 optical system (Bio-Rad) with 10min 95°C initial denaturation followed by 45 cycles containing a 15s step at 95°C and a second at 60°C for 1min. Data were quantified based on the Livak method ($2^{-\Delta\Delta Ct}$) [137] assuming 95% efficiency [138, 139], normalized to the reference gene GAPDH for mRNA, and to the geometric mean between UTR and 5srRNA for DNA to present the difference in mean between populations relative to the adult one.

Statistical analysis. Unpaired parametric t tests were performed for $1.95^{-\Delta Ct}$ with GraphPad Prism v6.05, results considered significant different when $P < 0.0500$. Populations were assumed having equal standard deviation (SD), with n=5 for tissue samples and n=4 for cell samples. Data from samples run twice were combined as the arithmetic mean between their $1.95^{-\Delta Ct}$ relative to the mean $1.95^{-\Delta Ct}$ of the adult population of correlating plate, in the same manner samples from same population on different plates were combined relative to the adult population. Data are presented as column bars with mean + SD.

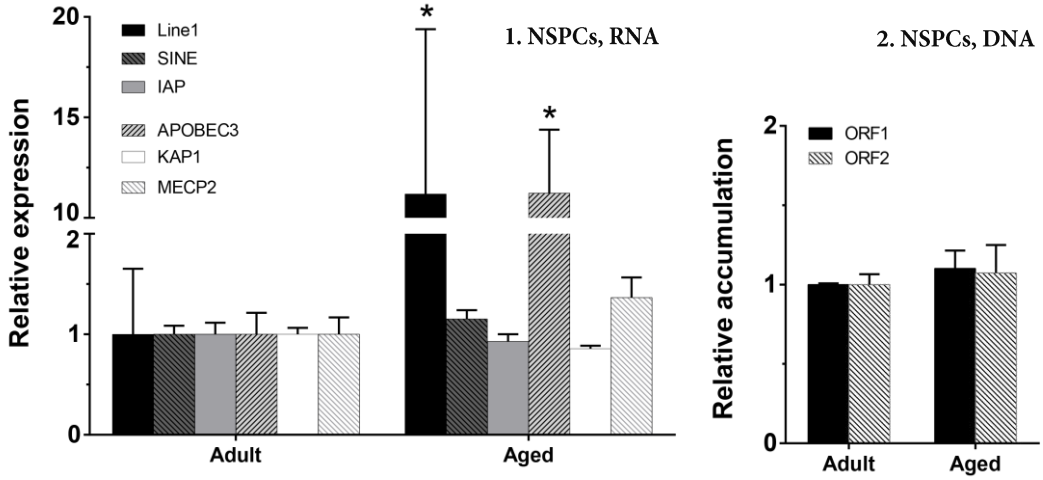

Figure 1. Increased expression of LINE1 and APOBEC3 in aged NSPCs. QPCR analysis of RNA expression of retroelements LINE1, SINE, IAP and regulators APOBEC3, KAP1 and MECP2 in adult and aged NSPCs grown as neurospheres and collected for analysis at passage 1 and 2. Data presented as means + SD, * indicates $P < 0.0500$, n=4.

Figure 2. QPCR analysis of primers, ORF1 and ORF2, detecting genomic L1 DNA in adult and aged NSPCs grown as neurospheres and collected for analysis at passage 1 and 2. Data presented as means + SD, * indicates $P < 0.0500$, n=4.

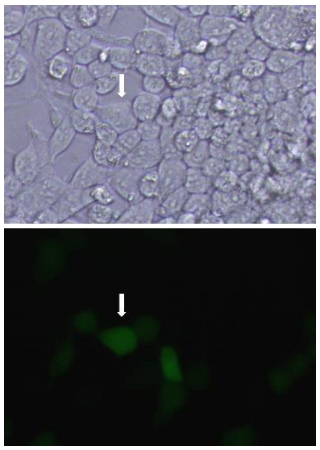

Figure 3. Validation of pL1RP-EGFP plasmid. 293t cells were transfected with EF06R plasmid and analyzed for GFP+ cells that had retroevents.

Results

Higher LINE1 expression in aged NSPCs. In order to explore if age dependent decline of neurogenesis could be connected to mechanisms involving retrotranspositioning, we examined the expression of LINE1, SINE and IAP retrotransposons as well as known regulators KAP1, APOBEC3 and MECP2 in adult and aged NSPCs. We used a previously published qPCR assay [90, 117, 140-143] to quantify expression in adult and aged neurospheres. Since early but not late passage neurospheres have been shown to replicate age related differences in neurogenesis [38] we collected RNA during the two first passages. Strikingly, we found that expression of L1 was increased 11.18 fold in aged compared to adult NSPCs (Figure 1). In contrast, we could not detect any difference in expression of SINE or IAP elements. In order to find a possible mechanism for the increased expression of L1, we analyzed known retrotransposon regulators. We did not detect any significant differences in expression of KAP1 or MECP2 (Figure 1), however, there was an 11.23 fold higher expression of APOBEC3 in aged compared to adult NSPCs (Figure 1). Taken together these data indicate that L1 expression is increased in aged NSPCs, and that in response the negative regulator APOBEC3 is concomitantly increased.

No evidence for increased L1 levels in aged NSPCs. Since we detected increased expression of L1 we decided to investigate if this boost also leads to increased insertions resulting in higher levels of mobile elements in the genome of NSPCs. DNA samples extracted from the first two passages of adult and aged neurospheres were analyzed with qPCR, where two set of primers, ORF1 and ORF2, detecting L1 genome was used [90, 117]. In contrast to the finding of increased L1 expression we could not detect any significant increase of total genomic levels of L1 (Figure 2). These data suggests that the increased expression of L1 does not lead to any substantial increase in total genomic LINE1 levels.

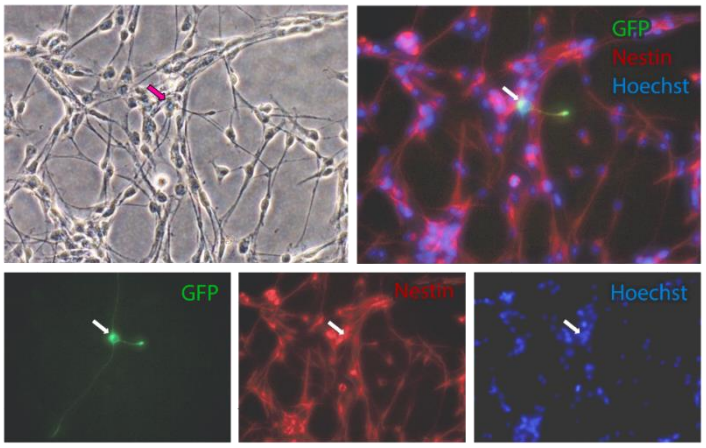

Figure 4. Integration of L1 in adult NSPCs. Neurosphere cells were nucleofected with EF06R plasmid and analyzed by immunocytochemstry for GFP and NSCP marker Nestin. Arrow indicate GFP/Nestin double labeled cell that underwent retrotransposition.

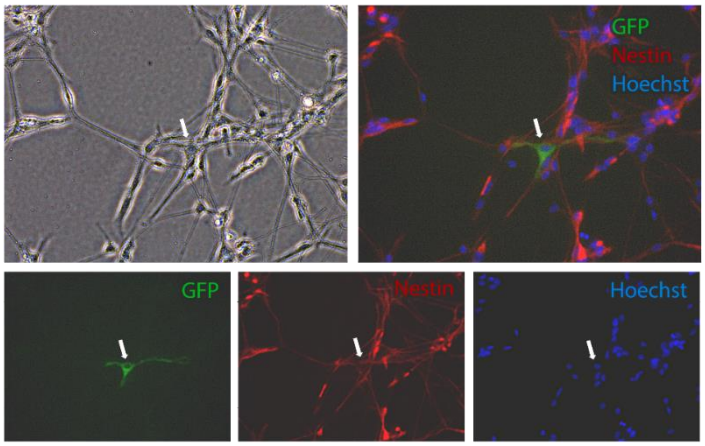

Figure 5. Integration of L1 in aged NSPCs. Neurosphere cells were nucleofected with EF06R plasmid and analyzed by immunocytochemstry for GFP and NSCP marker Nestin. Arrow indicate GFP/Nestin double labeled cell that underwent retrotransposition.

L1 retroevents occurs in adult SVZ derived NSPCs. Previous studies have indicated that retro elements are active in human fetal and rat hippocampal NSPCs [89, 115, 134], and since we did not find any proof of increased L1 levels in the genome of aged SVZ derived NSPCs despite increased expression we wanted to analyze retrotransposon activity in real time. For this purpose we used a reporter system based on a plasmid containing the pL1$_{RP}$-EGFP cassette where GFP is only expressed after a successful round of retrotransposition and insertion in the genome. We first validated the reporter in HEK 293t cells, which is known to have high levels of retrotransposition. In deed we could detect a large amount of GFP+ cells following transfection with pL1$_{RP}$-EGFP, indicating insertions (Figure 3). We then used nucleofection to transfect pL1$_{RP}$-EGFP into adult and aged NSPCs grown as monolayers and cultured them for different timespans. We could detect rare GFP positive cells in the cultures both after 12 days, 15 days and 17 days (passaged once) (Figure 4, 5). We confirmed that the GFP+ cells were NSPCs and not differentiated cells by immunofluorescence against the NSPC marker Nestin. Due to the extremely low ratio of GFP+ cells, around one per $1{\cdot}10^6$ transfected cells, no reliable statistical analysis were made. However, we did detect relatively more GFP positive cells in aged NSPC cultures. These findings propose that L1 transposition take place in adult murine NSPCs and actually are integrated in the genome, although very rarely.

L1 expression in NSPCs in vitro does not mirror retrotransposon activity in the brain. Since we detected real time retroevents in NSPCs and higher expression of L1 in aged NSPCs, we wanted to study if this is also reflected in the brain in vivo. Therefore we dissected regions, with or without neurogenesis, from adult and aged brain and analyzed expression of retroelements and regulators as well as genomic levels of retroelements. Tissue samples from the olfactory bulb (OB), hipocampus (HC) and cerebral cortex (CTX), were collected to compare expression of LINE1, SINE, IAP, KAP1, APOBEC3 and MECP2 as well as genomic L1 ORF1 and ORF2 between adult and aged mice using qPCR as described above. Interestingly, we could not detect any differences between the groups in any of the samples (Figure 6, 7). Indicating that the observed changes in vitro is not detectable in vivo.

Discussion

We show here for the first time that L1 retroevents take place in adult murine SVZ NSPCs, and that expression of L1 and APOBEC3 are substantially increased in aged compared to adult NSPCs but without any evidence for accumulation of new genomic insertions. Taken together, this provides support for a possible signaling mechanism that increases with advancing age and influence L1 and APOBEC3 simultaneously, where APOBEC3 is triggered in time to quench L1 and deny insertion before a full retrotransposition cycle is completed. In accordance with our findings, increased expression of LINE-like as well as LTR retrotransposons in old flies [144] and APOBEC2 in aged rat skeletal muscle [145] has earlier been reported. Interestingly, interferon alfa (IFN-α) signaling, suggested to be triggered by L1 [146, 147] and up-regulate APOBEC3 [148], has also recently been linked with aging in the brain [149] and could be a potential mechanistic explanation to our findings. It would therefore be crucial in future studies to investigate IFN-α expression in aged NSPCs. It would also be interesting to further investigate AID that is closely related to APOBEC3 [87, 150], and other factors connected with both L1 and APOBEC3.

We did not find any evidence for increased retroelement insertion in the genome of aged NSPCs. However, given the difficulties in reliably quantify genomic insertions based on qPCR one cannot rule out the possibility that also insertions are affected by aging, although below the detection limit in our system. It is also possible that the activity is only transferred to insertions in differentiating progeny. To further investigate the matter, specific primers that only detects still active L1 elements [61, 151] present in the genome could be chosen, and used together with the more efficient Taqman

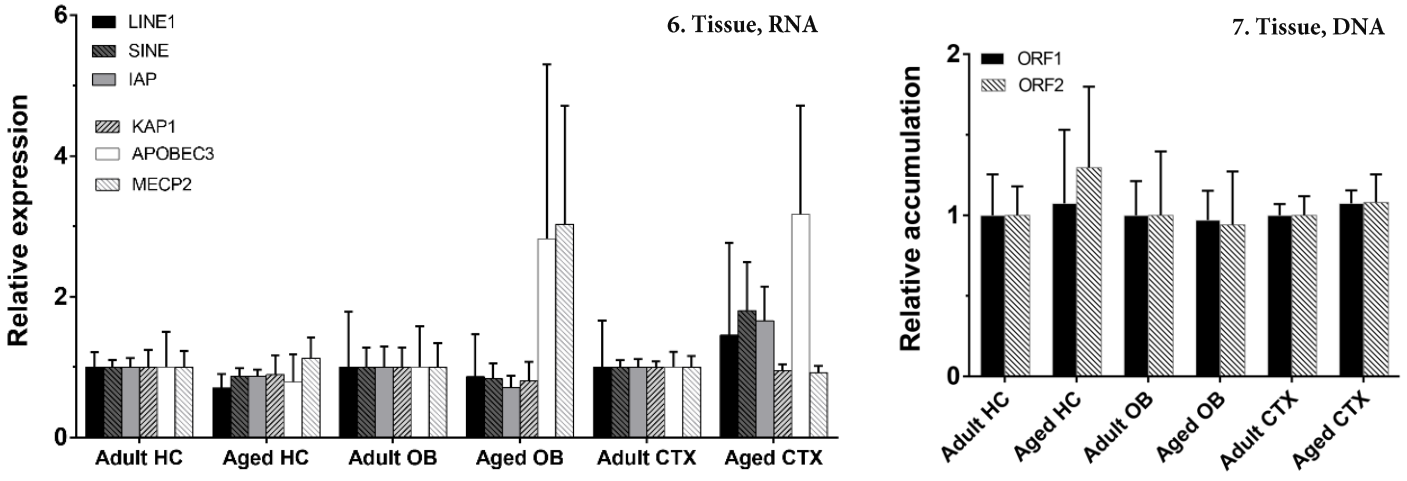

Figure 6. QPCR analysis of RNA expression of retroelements LINE1, SINE, IAP and regulators APOBEC3, KAP1 and MECP2 in adult and aged tissue collected from hippocampus (HC), olfactory bulb (OB) and cerebral cortex (CTX). Data presented as means + SD, * indicates $P < 0.0500$, n=4.

Figure 7. QPCR analysis of primers, ORF1 and ORF2, detecting genomic L1 DNA in adult and aged tissue collected from hippocampus (HC), olfactory bulb (OB) and cerebral cortex (CTX). Data presented as means + SD, * indicates $P < 0.0500$, n=4.

probes for qPCR instead of SYBR green [152, 153] on both NSPCs and differentiated progeny. This would eliminate the huge background of evolutionary accumulated inactive elements, incapable of insertions, that build up about 10% of the mouse genome [61]. Another delicate method is genome sequencing, which would allow quantification and visualization at which loci possible new insertions have ended up, and look for different patterns of transposon mediated mutations. This has earlier been carried out in human brain tissue on single cell level [125, 154]. More specific primers could be used in the same manner to examine which class of L1 that are expressed, and if it's a certain subtype that increase with aging. It would also be interesting to see if any specific group of APOBEC3 is expressed to a higher extent in elderly human, since not all hinder L1 activity [82, 155, 156], and investigate if any possible up regulated types have an inhibitory effect on other expressions that are crucial for maintaining inceptive cell functions.

Our findings, using the L1-reporter, does indicate that retroevents occurs in adult and aged mouse NSPCs. These results are in line with findings from human neural stem cells [115]. However, we only detected very rare events (apx. $1/1{\cdot}10^6$ cells) which is at least 10 fold less compared to those reported for human NSPCs, and did not allow for a reliable comparison between adult and aged cells. This discrepancy might be explained by species differences but also highlights the need for more sensitive methods to detect real time retro events. An intriguing possibility would be to use the Crispr/Cas9 method to visualize genomic retroelement sequences.

Our results imply that the changes observed in vitro isn't detectable, or doesn't transpire in vivo. We dissected brain regions with or without NSPCs and ongoing neurogenesis that should in theory reveal, if they occur, the differences observed in vitro. However, tissue samples obviously lack cellular resolution and potential differences in a specific cell type might be masked by the heterogeneity of the samples. An interesting experiment would be to carefully sub-dissect SVZ and dentate gyrus in order to enrich for NSPCs or even more elegant, but technically difficult, to sort single NSPCs and differentiated progeny and perform a similar analysis. Indeed, analysis of neurons sorted by FACS have been shown to accumulate transposons at a higher rate than other cell types [89, 115, 117, 125]. Nevertheless, if a higher amount of insertions were detected in NSPCs, it would be reasonable that also a higher accumulation of transposons were found in the progeny in vivo, such as in the olfactory bulb and hippocampus analyzed in this study.

Here we have for the first time described that retrotransposition occurs in adult and aged SVZ NSPCs and that expression of L1 and its inhibitor APOBEC3 is concomitantly increased in adult compared to aged NSPCs without leading to increased insertions. These findings are potentially very important in order to understand age related decrease in neurogenesis. Further studies to validate these findings and to explore the functional consequences of increased retrotransposon activity and insertions in NSPCs are highly warranted.

Acknowledgments

We thank the members of Ahlenius and Kokaia groups for scientific feedback and shared supplies, Giedre Miskinyte for supplying 293t cells, and Johan Jakobsson for helpful discussions.

References

1. Seaberg, R.M. and D. van der Kooy, *Stem and progenitor cells: the premature desertion of rigorous definitions.* Trends Neurosci, 2003. **26**(3): p. 125-31.
2. Merkle, F.T. and A. Alvarez-Buylla, *Neural stem cells in mammalian development.* Curr Opin Cell Biol, 2006. **18**(6): p. 704-9.
3. Weiner, L.P., *Definitions and criteria for stem cells.* Methods Mol Biol, 2008. **438**: p. 3-8.
4. Bonaguidi, M.A., et al., *In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics.* Cell, 2011. **145**(7): p. 1142-55.
5. Giachino, C. and V. Taylor, *Notching up neural stem cell homogeneity in homeostasis and disease.* Front Neurosci, 2014. **8**: p. 32.
6. Gage, F.H. and S. Temple, *Neural stem cells: generating and regenerating the brain.* Neuron, 2013. **80**(3): p. 588-601.
7. Goritz, C. and J. Frisen, *Neural stem cells and neurogenesis in the adult.* Cell Stem Cell, 2012. **10**(6): p. 657-9.
8. Kriegstein, A. and A. Alvarez-Buylla, *The glial nature of embryonic and adult neural stem cells.* Annu Rev Neurosci, 2009. **32**: p. 149-84.
9. Gage, F.H., *Mammalian neural stem cells.* Science, 2000. **287**(5457): p. 1433-8.
10. Maslov, A.Y., et al., *Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice.* J Neurosci, 2004. **24**(7): p. 1726-33.
11. Doetsch, F., J.M. Garcia-Verdugo, and A. Alvarez-Buylla, *Regeneration of a germinal layer in the adult mammalian brain.* Proc Natl Acad Sci U S A, 1999. **96**(20): p. 11619-24.
12. Reynolds, B.A. and S. Weiss, *Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system.* Science, 1992. **255**(5052): p. 1707-10.
13. Richards, L.J., T.J. Kilpatrick, and P.F. Bartlett, *De novo generation of neuronal cells from the adult mouse brain.* Proc Natl Acad Sci U S A, 1992. **89**(18): p. 8591-5.
14. Morshead, C.M., C.G. Craig, and D. van der Kooy, *In vivo clonal analyses reveal the properties of endogenous neural stem cell proliferation in the adult mammalian forebrain.* Development, 1998. **125**(12): p. 2251-61.
15. Kukekov, V.G., et al., *Multipotent stem/progenitor cells with similar properties arise from two neurogenic regions of adult human brain.* Exp Neurol, 1999. **156**(2): p. 333-44.
16. Fuentealba, L.C., K. Obernier, and A. Alvarez-Buylla, *Adult neural stem cells bridge their niche.* Cell Stem Cell, 2012. **10**(6): p. 698-708.
17. Gato, A. and M.E. Desmond, *Why the embryo still matters: CSF and the neuroepithelium as interdependent regulators of embryonic brain growth, morphogenesis and histiogenesis.* Dev Biol, 2009. **327**(2): p. 263-72.
18. Doetsch, F. and A. Alvarez-Buylla, *Network of tangential pathways for neuronal migration in adult mammalian brain.* Proc Natl Acad Sci U S A, 1996. **93**(25): p. 14895-900.
19. Shapiro, E.M., et al., *Magnetic resonance imaging of the migration of neuronal precursors generated in the adult rodent brain.* Neuroimage, 2006. **32**(3): p. 1150-7.
20. Lois, C. and A. Alvarez-Buylla, *Long-distance neuronal migration in the adult mammalian brain.* Science, 1994. **264**(5162): p. 1145-8.
21. Lledo, P.M., M. Alonso, and M.S. Grubb, *Adult neurogenesis and functional plasticity in neuronal circuits.* Nature Reviews Neuroscience, 2006. **7**(3): p. 179-193.
22. Rochefort, C., et al., *Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory.* Journal of Neuroscience, 2002. **22**(7): p. 2679-2689.
23. Rochefort, C. and P.M. Lledo, *Short-term survival of newborn neurons in the adult olfactory bulb after exposure to a complex odor environment.* European Journal of Neuroscience, 2005. **22**(11): p. 2863-2870.
24. Zhao, C., et al., *Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus.* J Neurosci, 2006. **26**(1): p. 3-11.

25. Lu, B. and J.H. Chang, *Regulation of neurogenesis by neurotrophins: implications in hippocampus-dependent memory.* Neuron Glia Biol, 2004. **1**(4): p. 377-84.
26. Winocur, G., et al., *Inhibition of neurogenesis interferes with hippocampus-dependent memory function.* Hippocampus, 2006. **16**(3): p. 296-304.
27. Kirkwood, T.B., *Understanding the odd science of aging.* Cell, 2005. **120**(4): p. 437-47.
28. Hamilton, L.K., et al., *Aging and neurogenesis in the adult forebrain: what we have learned and where we should go from here.* Eur J Neurosci, 2013. **37**(12): p. 1978-86.
29. Weissman, I.L., *Stem cells: units of development, units of regeneration, and units in evolution.* Cell, 2000. **100**(1): p. 157-68.
30. Li, L. and H. Clevers, *Coexistence of quiescent and active adult stem cells in mammals.* Science, 2010. **327**(5965): p. 542-5.
31. Rando, T.A., *Stem cells, ageing and the quest for immortality.* Nature, 2006. **441**(7097): p. 1080-6.
32. Liu, L. and T.A. Rando, *Manifestations and mechanisms of stem cell aging.* J Cell Biol, 2011. **193**(2): p. 257-66.
33. Medvedev, Z.A., *An attempt at a rational classification of theories of ageing.* Biol Rev Camb Philos Soc, 1990. **65**(3): p. 375-98.
34. Behrens, A., et al., *Impact of genomic damage and ageing on stem cell function.* Nat Cell Biol, 2014. **16**(3): p. 201-7.
35. Bailey, K.J., A.Y. Maslov, and S.C. Pruitt, *Accumulation of mutations and somatic selection in aging neural stem/progenitor cells.* Aging Cell, 2004. **3**(6): p. 391-7.
36. Molofsky, A.V., et al., *Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing.* Nature, 2006. **443**(7110): p. 448-52.
37. Luo, J., et al., *The aging neurogenic subventricular zone.* Aging Cell, 2006. **5**(2): p. 139-52.
38. Ahlenius, H., et al., *Neural stem and progenitor cells retain their potential for proliferation and differentiation into functional neurons despite lower number in aged brain.* J Neurosci, 2009. **29**(14): p. 4408-19.
39. Jin, K., et al., *Neurogenesis and aging: FGF-2 and HB-EGF restore neurogenesis in hippocampus and subventricular zone of aged mice.* Aging Cell, 2003. **2**(3): p. 175-83.
40. Shetty, A.K., B. Hattiangady, and G.A. Shetty, *Stem/progenitor cell proliferation factors FGF-2, IGF-1, and VEGF exhibit early decline during the course of aging in the hippocampus: role of astrocytes.* Glia, 2005. **51**(3): p. 173-86.
41. Ferron, S.R., et al., *Telomere shortening in neural stem cells disrupts neuronal differentiation and neuritogenesis.* J Neurosci, 2009. **29**(46): p. 14394-407.
42. Ferron, S., et al., *Telomere shortening and chromosomal instability abrogates proliferation of adult but not embryonic neural stem cells.* Development, 2004. **131**(16): p. 4059-70.
43. Luo, J., et al., *Subventricular zone-mediated ependyma repair in the adult mammalian brain.* J Neurosci, 2008. **28**(14): p. 3804-13.
44. Enwere, E., et al., *Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination.* J Neurosci, 2004. **24**(38): p. 8354-65.
45. Tropepe, V., et al., *Transforming growth factor-alpha null and senescent mice show decreased neural progenitor cell proliferation in the forebrain subependyma.* J Neurosci, 1997. **17**(20): p. 7850-9.
46. Kuhn, H.G., H. Dickinson-Anson, and F.H. Gage, *Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation.* J Neurosci, 1996. **16**(6): p. 2027-33.
47. Hattiangady, B. and A.K. Shetty, *Aging does not alter the number or phenotype of putative stem/progenitor cells in the neurogenic region of the hippocampus.* Neurobiol Aging, 2008. **29**(1): p. 129-47.
48. Breckenridge, L.J., et al., *Localised degeneration occurs in aged mouse olfactory epithelium.* J Anat, 1997. **191 (Pt 1)**: p. 151-4.
49. Lander, E.S., et al., *Initial sequencing and analysis of the human genome.* Nature, 2001. **409**(6822): p. 860-921.
50. McClintock, B., *Induction of Instability at Selected Loci in Maize.* Genetics, 1953. **38**(6): p. 579-99.
51. Mc, C.B., *The origin and behavior of mutable loci in maize.* Proc Natl Acad Sci U S A, 1950. **36**(6): p. 344-55.
52. Mc, C.B., *Chromosome organization and genic expression.* Cold Spring Harb Symp Quant Biol, 1951. **16**: p. 13-47.
53. Britten, R.J. and D.E. Kohne, *Repeated sequences in DNA. Hundreds of thousands of copies of DNA sequences have been incorporated into the genomes of higher organisms.* Science, 1968. **161**(3841): p. 529-40.
54. Agrawal, A., Q.M. Eastman, and D.G. Schatz, *Transposition mediated by RAG1 and RAG2 and its implications for the evolution of the immune system.* Nature, 1998. **394**(6695): p. 744-51.
55. Beck, C.R., et al., *LINE-1 elements in structural variation and disease.* Annu Rev Genomics Hum Genet, 2011. **12**: p. 187-215.
56. Feschotte, C., *Transposable elements and the evolution of regulatory networks.* Nat Rev Genet, 2008. **9**(5): p. 397-405.
57. Levis, R.W., et al., *Transposons in place of telomeric repeats at a Drosophila telomere.* Cell, 1993. **75**(6): p. 1083-93.
58. Doolittle, W.F. and C. Sapienza, *Selfish genes, the phenotype paradigm and genome evolution.* Nature, 1980. **284**(5757): p. 601-3.
59. Hickey, D.A., *Selfish DNA: a sexually-transmitted nuclear parasite.* Genetics, 1982. **101**(3-4): p. 519-31.
60. Orgel, L.E., F.H. Crick, and C. Sapienza, *Selfish DNA.* Nature, 1980. **288**(5792): p. 645-6.
61. Goodier, J.L., et al., *A novel active L1 retrotransposon subfamily in the mouse.* Genome Research, 2001. **11**(10): p. 1677-1685.
62. Cordaux, R. and M.A. Batzer, *The impact of retrotransposons on human genome evolution.* Nature Reviews Genetics, 2009. **10**(10): p. 691-703.
63. Finnegan, D.J., *Retrotransposons.* Curr Biol, 2012. **22**(11): p. R432-7.
64. Redei, G.P. and SpringerLink (Online service), *Encyclopedia of genetics, genomics, proteomics, and informatics*, in *Springer reference.* 2008, Springer,: New York? p. 2 v.
65. Kazazian, H.H., Jr., *Mobile elements: drivers of genome evolution.* Science, 2004. **303**(5664): p. 1626-32.
66. Mills, R.E., et al., *Which transposable elements are active in the human genome?* Trends Genet, 2007. **23**(4): p. 183-91.
67. Dewannieux, M., C. Esnault, and T. Heidmann, *LINE-mediated retrotransposition of marked Alu sequences.* Nat Genet, 2003. **35**(1): p. 41-8.
68. Hancks, D.C., et al., *Retrotransposition of marked SVA elements by human L1s in cultured cells.* Hum Mol Genet, 2011. **20**(17): p. 3386-400.
69. Kuff, E.L. and K.K. Lueders, *The intracisternal A-particle gene family: structure and functional aspects.* Adv Cancer Res, 1988. **51**: p. 183-276.
70. Dewannieux, M., et al., *Identification of autonomous IAP LTR retrotransposons mobile in mammalian cells.* Nature Genetics, 2004. **36**(5): p. 534-539.
71. Dombroski, B.A., et al., *Isolation of an Active Human Transposable Element.* Science, 1991. **254**(5039): p. 1805-1808.
72. Kolosha, V.O. and S.L. Martin, *In vitro properties of the first ORF protein from mouse LINE-1 support its role in ribonucleoprotein particle formation during retrotransposition.* Proc Natl Acad Sci U S A, 1997. **94**(19): p. 10155-60.
73. Kolosha, V.O. and S.L. Martin, *High-affinity, non-sequence-specific RNA binding by the open reading frame 1 (ORF1) protein from long interspersed nuclear element 1 (LINE-1).* J Biol Chem, 2003. **278**(10): p. 8112-7.
74. Martin, S.L. and F.D. Bushman, *Nucleic acid chaperone activity of the ORF1 protein from the mouse LINE-1 retrotransposon.* Mol Cell Biol, 2001. **21**(2): p. 467-75.
75. Feng, Q., et al., *Human L1 retrotransposon encodes a conserved endonuclease required for retrotransposition.* Cell, 1996. **87**(5): p. 905-16.
76. Mathias, S.L., et al., *Reverse transcriptase encoded by a human transposable element.* Science, 1991. **254**(5039): p. 1808-10.
77. Dombroski, B.A., et al., *An in vivo assay for the reverse transcriptase of human retrotransposon L1 in Saccharomyces cerevisiae.* Mol Cell Biol, 1994. **14**(7): p. 4485-92.
78. Singer, T., et al., *LINE-1 retrotransposons: mediators of somatic variation in neuronal genomes?* Trends Neurosci, 2010. **33**(8): p. 345-54.
79. Beck, C.R., et al., *LINE-1 Retrotransposition Activity in Human Genomes.* Cell, 2010. **141**(7): p. 1159-U110.
80. Brouha, B., et al., *Hot L1s account for the bulk of retrotransposition in the human population.* Proceedings of the National Academy of Sciences of the United States of America, 2003. **100**(9): p. 5280-5285.
81. Kines, K.J., et al., *Potential for genomic instability associated with retrotranspositionally-incompetent L1 loci.* Nucleic Acids Res, 2014. **42**(16): p. 10488-502.

82. Muckenfuss, H., et al., *APOBEC3 proteins inhibit human LINE-1 retrotransposition.* Journal of Biological Chemistry, 2006. **281**(31): p. 22161-22172.
83. Kinomoto, M., et al., *All APOBEC3 family proteins differentially inhibit LINE-1 retrotransposition.* Nucleic Acids Research, 2007. **35**(9): p. 2955-2964.
84. Harris, R.S., et al., *DNA deamination mediates innate immunity to retroviral infection.* Cell, 2003. **113**(6): p. 803-9.
85. Zhang, H., et al., *The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA.* Nature, 2003. **424**(6944): p. 94-8.
86. Mangeat, B., et al., *Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts.* Nature, 2003. **424**(6944): p. 99-103.
87. Macduff, D.A. and R.S. Harris, *Directed DNA deamination by AID/APOBEC3 in immunity.* Curr Biol, 2006. **16**(6): p. R186-9.
88. MacDuff, D.A., Z.L. Demorest, and R.S. Harris, *AID can restrict L1 retrotransposition suggesting a dual role in innate and adaptive immunity.* Nucleic Acids Research, 2009. **37**(6): p. 1854-1867.
89. Muotri, A.R., et al., *Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition.* Nature, 2005. **435**(7044): p. 903-10.
90. Muotri, A.R., et al., *L1 retrotransposition in neurons is modulated by MeCP2.* Nature, 2010. **468**(7322): p. 443-6.
91. Meehan, R.R., J.D. Lewis, and A.P. Bird, *Characterization of MeCP2, a vertebrate DNA binding protein with affinity for methylated DNA.* Nucleic Acids Res, 1992. **20**(19): p. 5085-92.
92. Nan, X.S., F.J. Campoy, and A. Bird, *MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin.* Cell, 1997. **88**(4): p. 471-481.
93. Jones, P.L., et al., *Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription.* Nature Genetics, 1998. **19**(2): p. 187-191.
94. Nan, X., et al., *Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex.* Nature, 1998. **393**(6683): p. 386-9.
95. Fasching, L., et al., *TRIM28 Represses Transcription of Endogenous Retroviruses in Neural Progenitor Cells.* Cell Rep, 2015. **10**(1): p. 20-8.
96. Rowe, H.M., et al., *KAP1 controls endogenous retroviruses in embryonic stem cells.* Nature, 2010. **463**(7278): p. 237-40.
97. Rowe, H.M., et al., *TRIM28 repression of retrotransposon-based enhancers is necessary to preserve transcriptional dynamics in embryonic stem cells.* Genome Res, 2013. **23**(3): p. 452-61.
98. Wolf, D. and S.P. Goff, *Embryonic stem cells use ZFP809 to silence retroviral DNAs.* Nature, 2009. **458**(7242): p. 1201-4.
99. Thomas, J.H. and S. Schneider, *Coevolution of retroelements and tandem zinc finger genes.* Genome Res, 2011. **21**(11): p. 1800-12.
100. Sripathy, S.P., J. Stevens, and D.C. Schultz, *The KAP1 corepressor functions to coordinate the assembly of de novo HP1-demarcated microenvironments of heterochromatin required for KRAB zinc finger protein-mediated transcriptional repression.* Molecular and Cellular Biology, 2006. **26**(22): p. 8623-8638.
101. Schultz, D.C., et al., *SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins.* Genes & Development, 2002. **16**(8): p. 919-932.
102. Wiznerowicz, M., et al., *The Kruppel-associated box repressor domain can trigger de Novo promoter methylation during mouse early embryogenesis.* Journal of Biological Chemistry, 2007. **282**(47): p. 34535-34541.
103. Ellis, J., A. Hotta, and M. Rastegar, *Retrovirus silencing by an epigenetic TRIM.* Cell, 2007. **131**(1): p. 13-14.
104. Rowe, H.M. and D. Trono, *Dynamic control of endogenous retroviruses during development.* Virology, 2011. **411**(2): p. 273-87.
105. Malone, C.D. and G.J. Hannon, *Small RNAs as guardians of the genome.* Cell, 2009. **136**(4): p. 656-68.
106. Yang, N. and H.H. Kazazian, Jr., *L1 retrotransposition is suppressed by endogenously encoded small interfering RNAs in human cultured cells.* Nat Struct Mol Biol, 2006. **13**(9): p. 763-71.
107. Carmell, M.A., et al., *MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline.* Developmental Cell, 2007. **12**(4): p. 503-514.
108. Aravin, A.A., et al., *A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice.* Molecular Cell, 2008. **31**(6): p. 785-799.
109. Stetson, D.B., et al., *Trex1 prevents cell-intrinsic initiation of autoimmunity.* Cell, 2008. **134**(4): p. 587-98.
110. Coufal, N.G., et al., *Ataxia telangiectasia mutated (ATM) modulates long interspersed element-1 (L1) retrotransposition in human neural stem cells.* Proc Natl Acad Sci U S A, 2011. **108**(51): p. 20382-7.
111. Kuwabara, T., et al., *Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis.* Nature Neuroscience, 2009. **12**(9): p. 1097-U6.
112. Yang, N., et al., *An important role for RUNX3 in human L1 transcription and retrotransposition.* Nucleic Acids Research, 2003. **31**(16): p. 4929-4940.
113. Athanikar, J.N., R.M. Badge, and J.V. Moran, *A YY1-binding site is required for accurate human LINE-1 transcription initiation.* Nucleic Acids Research, 2004. **32**(13): p. 3846-3855.
114. Tchenio, T., J.F. Casella, and T. Heidmann, *Members of the SRY family regulate the human LINE retrotransposons.* Nucleic Acids Research, 2000. **28**(2): p. 411-415.
115. Coufal, N.G., et al., *L1 retrotransposition in human neural progenitor cells.* Nature, 2009. **460**(7259): p. 1127-31.
116. Van Meter, M., et al., *SIRT6 represses LINE1 retrotransposons by ribosylating KAP1 but this repression fails with stress and age.* Nat Commun, 2014. **5**: p. 5011.
117. Bundo, M., et al., *Increased l1 retrotransposition in the neuronal genome in schizophrenia.* Neuron, 2014. **81**(2): p. 306-13.
118. Li, W.H., et al., *Activation of transposable elements during aging and neuronal decline in Drosophila.* Nature Neuroscience, 2013. **16**(5): p. 529-+.
119. Kaer, K. and M. Speek, *Retroelements in human disease.* Gene, 2013. **518**(2): p. 231-41.
120. Belancio, V.P., D.J. Hedges, and P. Deininger, *Mammalian non-LTR retrotransposons: for better or worse, in sickness and in health.* Genome Res, 2008. **18**(3): p. 343-58.
121. Bernard, V., et al., *Exon deletions and intragenic insertions are not rare in ataxia with oculomotor apraxia 2.* BMC Med Genet, 2009. **10**: p. 87.
122. Mine, M., et al., *A large genomic deletion in the PDHX gene caused by the retrotranspositional insertion of a full-length LINE-1 element.* Hum Mutat, 2007. **28**(2): p. 137-42.
123. Maxwell, P.H., W.C. Burhans, and M.J. Curcio, *Retrotransposition is associated with genome instability during chronological aging.* Proceedings of the National Academy of Sciences of the United States of America, 2011. **108**(51): p. 20376-20381.
124. Iskow, R.C., et al., *Natural mutagenesis of human genomes by endogenous retrotransposons.* Cell, 2010. **141**(7): p. 1253-61.
125. Evrony, G.D., et al., *Single-neuron sequencing analysis of L1 retrotransposition and somatic mutation in the human brain.* Cell, 2012. **151**(3): p. 483-96.
126. Fischer, H.G., et al., *Changes in neuronal DNA content variation in the human brain during aging.* Aging Cell, 2012. **11**(4): p. 628-33.
127. Lee, K.H., et al., *Age-dependent and tissue-specific structural changes in the C57BL/6J mouse genome.* Exp Mol Pathol, 2012. **93**(1): p. 167-72.
128. Gasior, S.L., et al., *The human LINE-1 retrotransposon creates DNA double-strand breaks.* J Mol Biol, 2006. **357**(5): p. 1383-93.
129. Belgnaoui, S.M., et al., *Human LINE-1 retrotransposon induces DNA damage and apoptosis in cancer cells.* Cancer Cell International, 2006. **6**.
130. Farkash, E.A. and E.T.L. Prak, *DNA damage and L1 retrotransposition.* Journal of Biomedicine and Biotechnology, 2006.
131. Hedges, D.J. and V.P. Belancio, *Restless genomes humans as a model organism for understanding host-retrotransposable element dynamics.* Adv Genet, 2011. **73**: p. 219-62.
132. de Lau, L.M. and M.M. Breteler, *Epidemiology of Parkinson's disease.* Lancet Neurol, 2006. **5**(6): p. 525-35.
133. Nussbaum, R.L. and C.E. Ellis, *Alzheimer's disease and Parkinson's disease.* N Engl J Med, 2003. **348**(14): p. 1356-64.
134. Muotri, A.R., et al., *Environmental influence on L1 retrotransposons in the adult hippocampus.* Hippocampus, 2009. **19**(10): p. 1002-7.
135. Ahlenius, H. and Z. Kokaia, *Isolation and generation of neurosphere cultures from embryonic and adult mouse brain.* Methods Mol Biol, 2010. **633**: p. 241-52.
136. Farkash, E.A., et al., *Gamma radiation increases endonuclease-dependent L1 retrotransposition in a cultured cell assay.* Nucleic Acids Res, 2006. **34**(4): p. 1196-204.

137. Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.* Methods, 2001. **25**(4): p. 402-8.
138. Pfaffl, M.W., *A new mathematical model for relative quantification in real-time RT-PCR.* Nucleic Acids Res, 2001. **29**(9): p. e45.
139. Ramakers, C., et al., *Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data.* Neuroscience Letters, 2003. **339**(1): p. 62-66.
140. Nolan, T., R.E. Hands, and S.A. Bustin, *Quantification of mRNA using real-time RT-PCR.* Nature Protocols, 2006. **1**(3): p. 1559-1582.
141. Huggett, J., et al., *Real-time RT-PCR normalisation; strategies and considerations.* Genes and Immunity, 2005. **6**(4): p. 279-284.
142. Sachdeva, R., et al., *Tracking differentiating neural progenitors in pluripotent cultures using microRNA-regulated lentiviral vectors.* Proc Natl Acad Sci U S A, 2010. **107**(25): p. 11602-7.
143. Fadloun, A., et al., *Chromatin signatures and retrotransposon profiling in mouse embryos reveal regulation of LINE-1 by RNA.* Nat Struct Mol Biol, 2013. **20**(3): p. 332-8.
144. Li, W., et al., *Activation of transposable elements during aging and neuronal decline in Drosophila.* Nat Neurosci, 2013. **16**(5): p. 529-31.
145. I, P., et al., *Differential proteome analysis of aging in rat skeletal muscle.* Faseb Journal, 2005. **19**(6): p. 1143-+.
146. Mavragani, C.P. and M.K. Crow, *Activation of the type I interferon pathway in primary Sjogren's syndrome.* Journal of Autoimmunity, 2010. **35**(3): p. 225-231.
147. Mavragani, C.P., et al., *Endogenous line-1 retroviral elements in older patients with primary Sjogren's syndrome: Triggers of interferon-alpha production and B-cell activation?* Annals of the Rheumatic Diseases, 2007. **66**: p. A47-A47.
148. Chen, K., et al., *Alpha interferon potently enhances the anti-human immunodeficiency virus type 1 activity of APOBEC3G in resting primary CD4 T cells.* J Virol, 2006. **80**(15): p. 7645-57.
149. Baruch, K., et al., *Aging-induced type I interferon response at the choroid plexus negatively affects brain function.* Science, 2014. **346**(6205): p. 89-93.
150. Koito, A. and T. Ikeda, *Intrinsic restriction activity by AID/APOBEC family of enzymes against the mobility of retroelements.* Mob Genet Elements, 2011. **1**(3): p. 197-202.
151. Hardies, S.C., et al., *LINE-1 (L1) lineages in the mouse.* Mol Biol Evol, 2000. **17**(4): p. 616-28.
152. Bustin, S.A., *Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays.* J Mol Endocrinol, 2000. **25**(2): p. 169-93.
153. Smith, C.J. and A.M. Osborn, *Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology.* FEMS Microbiol Ecol, 2009. **67**(1): p. 6-20.
154. Baillie, J.K., et al., *Somatic retrotransposition alters the genetic landscape of the human brain.* Nature, 2011. **479**(7374): p. 534-7.
155. Wissing, S., et al., *Endogenous APOBEC3B restricts LINE-1 retrotransposition in transformed cells and human embryonic stem cells.* J Biol Chem, 2011. **286**(42): p. 36427-37.
156. Stenglein, M.D. and R.S. Harris, *APOBEC3B and APOBEC3F inhibit L1 retrotransposition by a DNA deamination-independent mechanism.* Journal of Biological Chemistry, 2006. **281**(25): p. 16837-16841.
157. Sanville, B., et al., *Adaptive evolution of Mus Apobec3 includes retroviral insertion and positive selection at two clusters of residues flanking the substrate groove.* PLoS Pathog, 2010. **6**: p. e1000974.
158. Spandidos, A., et al., *PrimerBank: a resource of human and mouse PCR primer pairs for gene expression detection and quantification.* Nucleic Acids Res, 2010. **38**(Database issue): p. D792-9.

Table1. Primers used for Quantitative RT-PCR analyses, reference genes applied for normalization are

cDNA	Primer sequence (Forward)	Primer sequence (Reverse)	References
APOBEC3	5'GACCATTCTGTCTGGGATGCA	5'TTCTAGTCACTTCATAGCACA	[157]
GAPDH (ref)	5'TCCATGACAACTTTGGCATTG	5'CAGTCTTCTGGGTGGCAGTGA	[142]
IAP	5'CTCCATGTGCTCTGCCTTCC	5'CCCCGTCCCTTTTTTAGGAGA	[143]
KAP1 (Trim28)	5'CGGAAATGTGAGCGTGTTCTC	5'CGGTAGCCAGCTGATGCAA	[97]
LINE1	5'AGTGCAGAGTTCTATCAGACCTTC	5'AACCTACTTGGTCAGGATGGATG	[143]
MECP2	5'ATGGTAGCTGGGATGTTAGGG	5'TGAGCTTTCTGATGTTTCTGCTT	[158] ID: 14149645a1
SINEB2	5'GAGCACCTGACTGCTCTTCC	5'ACACACCAGAAGAGGGCATC	[143]
gDNA			
ORF1	5'TGGAAGAGAGAATCTCAGGTGC	5'TTGTGCCGATGTTCTCTATGG	[117]
ORF2	5'CTGGCGAGGATGTGGAGAA	5'CCTGCAATCCCACCAACAAT	[90, 117]
5SsrRNA (ref)	5'ACGGCCATACCACCCTGAA	5'GGTCTCCCATCCAAGTACTAACCA	[90, 117]
5'UTR (ref)	5'TAAGAGAGCTTGCCAGCAGAGA	5'GCAGACCTGGGAGACAGATTCT	[90, 117]