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Research Article

Long-Non-Coding RNAs and HuR Interaction may Regulate Neural Stem Cell Differentiation

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Abstract

Lately increasing number of studies is enhancing a functional rule for the long-non-coding-RNAs (lncRNAs) in gene expression regulation at multiple levels: transcriptional, translational and post-translational processes. Interestingly, a key and essential role has been found in brain development, where some specific lncRNAs genetic ablation has shown to cause a strong modification of the mouse phenotype, but less is known regarding their molecular way of action in the brain and neural stem cells. Here, we have identified a pool of lncRNAs differentially regulated during neural stem cells (NSCs) differentiation and their binding to the RNA-binding-protein ELAV1/HuR, a component of a protein family involved in transcriptional regulation in the nervous system, the ELAVL family. We suggest that HuR interaction with lncRNAs must be regulatory for the neural differentiation process by showing that specific pharmacological inhibition leads to powerful differentiation impairment with enhanced stemness features.

ABBREVIATIONS

lncRNA: Long-Non-Coding RNA; HuR: Human Antigen R; NSC: Neural Stem Cell; MTX: Mitoxantrone

INTRODUCTION

Neural Stem Cells (NSC) are self-renewing multipotent cells able to generate all the main cell phenotypes in the nervous system; neurons, astrocytes and oligodendrocytes [1]. These cells where first identified as resident progenitors in the sub-ventricular zone (SVZ) and dentate gyrus of mouse brain [2], and later have found a strong use in cell therapy applied to neurodegenerative disorders [3,4]. In that context NSCsare described to act both in early and late disease rescue by releasing soluble factors, active on the recipient microenvironment exerting an anti-inflammatory action and also differentiating themselves into nervous system cellular components [5-9].

Many progresses has been made in understanding what regulates adult neural stem cell specification, proliferation and differentiation, but much remains to be determined.In particular concerning the field of investigations studying the long-non-coding RNA molecules (lncRNA) [10].

LncRNAs are defined as RNA molecules synthesized by the cell longer than 200bp with a gene expression regulatory ability. Several well-studied examples of lncRNAs suggest that they can operate through distinct modes, including as signals, scaffolds for protein-protein interactions, molecular decoys, and guides to target elements in the genome or transcriptome [11]. Several lncRNAs are involved in the specific regulation of somatic tissue stem cell renewal or differentiation. Some lncRNAs maintain the staminality state, while others promote a differentiation program. Their functions are often favored by protein partners that guide the ability to activate or repress gene expression or post-transcriptionally regulate other RNAs. LncRN as were found to be widely expressed during NSC differentiation [12], and their genetic ablation in mouse models resulted into strong brain development impairment [13].

HuR/ELAV1 (Human Antigen R; HuR) is a member of the RNA-binding-protein ELAV (embryonic lethal abnormal version) family, well studied for its role in RNA splicing and mRNA post-transcriptional regulation [14,15]. HuR has been recently correlated to stem cell pluripotency, not for its long known mRNA stabilization activity, but for its interaction with lncRNAs, such as, linc-MD1, which is involved in muscle development (lincMD1). HuR has been also reported to influence lincRNA-p21 decay in

mouse fibroblasts, where this interaction has been proposed to regulate the translation of a subset of target mRNAs [16,17].

Much less is known about the regulatory pathways where lncRNAs are involved, and their molecular, particularly, in NSCs differentiation. Here, we report the ability of the RNA-binding-protein HuR in binding some lncRNAs expressed during neural development.

Therefore, this study is focused on the investigation of a potential role for the HuR interaction with specific lncRNAs in NSCs challenged to differentiate *"in vitro"*. Here, we firstly report the HuR interaction with specific lncRNAs that play a basal role in mouse brain development and embryonic stem cells neural differentiation. Moreover, we show that specific inhibition of HuR binding to RNA molecules, by mitoxantrone treatment, leads to lncRNAs detachment, inhibition of neural differentiation and enhancement of stem cell phenotype [18-21].

MATERIALS AND METHODS

Primary cell isolation and differentiation

NSCs were obtained from 6 weeks old CD-1 albino mice; their isolation, growth and characterization were performed following methods described by Gritti et al. [21]. Briefly, cells were isolated from the sub ventricular zone (SVZ) of adult male mice (CD1) immediately after their killing by cervical dislocation. Brains were removed and tissues containing the SVZ region were dissected, transferred to Earl's Balanced Salt Solution (Life Technologies, Monza, Italy) containing 1 mg/ml papain (27 U/mg; Sigma-Aldrich, Milan, Italy), 0.2 mg/ml cysteine (Sigma-Aldrich), and 0.2 mg/ml EDTA (Sigma-Aldrich) and incubated for 45 min at 37°C on a rocking platform. Tissues were then transferred to DMEM–F12 medium (Euroclone, Milan, Italy), and mechanically dissociated with Pasteur pipette. Cells were collected by centrifugation (200xg, 10 min, room temperature), and resuspended in DMEM-F12 medium (Euroclone) containing 2 mm l-glutamine (Euroclone), 0.6% glucose (Sigma-Aldrich), 9.6 gm/ml putrescine (Sigma-Aldrich), 6.3 ng/ml progesterone (Sigma-Aldrich), 5.2 ng/ml sodium selenite (Sigma-Aldrich), 0.025 mg/ml insulin (Sigma-Aldrich), 0.1 mg/ml transferrin (Sigma-Aldrich), and 2 μg/ml heparin (sodium salt, grade II; Sigma-Aldrich), bFGF (human recombinant, 10 ng/mL; Life Technologies) and EGF (human recombinant, 20 ng/mL; Life Technologies). PM-NPCs precursors were maintained in culture in the cited above medium. Differentiation of PM-NPCs was performed by plating the dissociated stem cells at the density of 40,000 cells/cm^2 in presence of adhesion molecules (Matrigel™, BD Biosciences, MI, Italy), and bFGF (10ng/ml) for 48 hours, then cells were exposed to the same medium without bFGF, and the addition of foetal bovine serum (2% vol/vol; Euroclone) for the following 5 days as previously described. Then, the extent of differentiation was determined by immunocytochemical staining [22-28]. Where indicated cells were treated with Mitoxantrone 2nM (Sigma M6545) [29].

RNA immunoprecipitation (RIP)

RIP was performed as reported in Latorre et al 2012 (30). RNA was isolated from the different samples (immunoprecipitated anti-HuR, IgG and precleared input) by TriZol as manufacturer hasrecommended,retrotranscribed into cDNA by MBI-Fermentas kit and used as template for PCR analysis.

Primers

linc-Brn1a sense.: CATCGAGGGAGAGGGACAGAG antisense.: CCAAAGCACCATTTCATCACATCAG; linc-Brn1b sense.: TGCCAGCTTGGCTTGTACTC antisense.: GCTCCCAAAGGTTCCTGTGTC; linc-Cox2 sense.: CTTATTTAGGAGGGTGGGAAGTC antisense.: GTGGTGGAGCTAGTGTCTCTTAG;Fablsense.: GAGCTACCAGGGAGGTGAAG antisense.: AGCTCAAGGGTTCTATGATGAGAG; linc-Enc1 sense.:CCTCCCTGATCTCTTTGCTTCC antisense.: CGACCACTGGTTCTGCACTC; Fendrrsense.: GGCCACAGCGGTCAGTTAC antisense.: TCTGGTGGAGTCAGATCAAACG; Haunt sense.: CTCAACTGCTGAGCCGTATC antisense.: GGCACTGGGTGGTCAAATAATG; Hottipsense.: GGCTTTGGGCTGCATCTTTG antisense.: GTCCTTCCACGACAGGGATAC;Mdgtsense.: GGTCATCAGAATACGGAATCCAC antisense.: CCAGCCAAGAGTCTCAGTGAAAG; linc-p21 sense.: AGTAGGGTGTTGTTCAGTTGGTAG antisense.: TCACAGGTATGAGGTGCAGAC; Peril sense.: GAGACACCTTCACGGACATAC antisense.: GGTGGTGGGTAGTGCTTCTTC; Tug1 sense.: TGACTGGCCCAGAAGTTGTAAG antisense.: GCAAGCAGGTCTGTGAGACTATTC.

qReal time-PCR quantification of mRNAs

Total RNA from cultured cells and tissue samples were isolated using TRIzol Reagent (Invitrogen). RNA quality was ascertained using a spectrophotometer and visualized on agarose gel.

Total RNA (1 μg) was reverse transcribed using iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions. qRT-PCR was performed with an DNA engine OPTICON2 detection system (MJ Research) using iQ SYBR Green Supermix (Bio-Rad) and the primers reported above in the primers paragraph.

Primers

SOX2 antisense.:CATGTATAACATGATGGAGACGGAGCTGAA antisense.:TTACGCCGCTGCCCCCGGGACCATACCATG; OCT4 sense.:AAGGGCCTCCAGGTGGGCCTGGAATCGGACantisense.:CTCGTGCTCCTGCCTGGCCCTCAGGCTGCA; NANOG sense.:GACAAGGGCCCTGAGGAGGAGGAGAACAAGantisense.:TTATAGCTCAGGTTCAGAATGGAGGAGAGT; GFAP sense.:TCAATGACCGCTTTGCTAGCTACATCGAGAantisense.:GGGCACTGTTGGCCGTAAGCTGGTCCAGCC; Tuj1 sense.:TTCACCACCAGCGCCAGCACCCCTGTGACCantisense.:AATGTTCACGGAAGTGGCCGTGCTTGGGAG; fibrsense.:TGCCTGGGGACCTCGGTGCGCTGCACCGAAantisense.:GGTGTTCATGATGCGGTCGGGGTACTCCTC; CD44 sense.:ATCAGTCACAGACCTACCCAATTCCTTCGAantisense.:TCTTCTCGATGGTGGAGCCGCTGCTGACAT; nestinsense.:GGAGGCTGAGAACTCTCGCTTGCAGACACCantisense.:TATTAGGCAAGGGGGAAGAGAAGGATGTTG; 18S sense.:TTGACGGAAGGGCACCACCAGantisense.:CTCCTTAATGTCACGCACGATTTC.

Immunocytochemistry

Cells were seeded on acid-washed glass coverslips and maintained in the appropriate culture medium, and experimental conditions. Cells were fixed and stained accordingly to Latorre et al. 2012 [30].

The following antibodies were used: Anti-nestin (Millipore MAB353), Anti-HuR (Santa Cruz SC71290), Anti-b tubulin III TUJ-1 (Biolegend; MMS-435P), anti-MAP2 (Millipore Ab5622). 4,6, diamino-2-phenyl-l-indole di-hydrocloride (DAPI; SigmaD259542) was used as counter-staining.

Tissue collection and processing, histology and immunohistochemistry

Animals were anesthetized by i.p. injection of cloralium hydrate (Sigma-Aldrich) 4% in distilled water, and perfused with 4% paraformaldehyde in phosphate buffer (PB) 0.1 M pH 7.4 by transcardial perfusion. Brains were dissected andpost-fixed overnight in the same fixative, cryoprotected with 30% sucrose (Sigma-Aldrich), quickly frozen, stored at -80°C, and sectioned by means of a cryostat (Leica). Sections were rinsed with PBS (Euroclone), treated with blocking solution (Life-Technologies) and incubated with Anti-HuR primary antibody (Santa Cruz; SC71290) overnight at 4°C. After treatment with primary antibodies, the sections were washed with PBS and incubated with appropriate secondary antibodies (Alexa Fluor® 488, Molecular Probes®, Life Technologies) for 2 hours at room temperature. Sections were washed in PBS, nuclei were stained with DAPI (1ug/ml final concentration, 10 minutes at room temperature), mounted using the FluorSave Reagent (Calbiochem, Merck Chemical, Darmstadt, Germany), and analyzed by confocal microscopy (Leica TSC2; Leica Microsystems). In control determinations, primary antibody was omitted and replaced with equivalent concentrations of unrelated IgG of the same subclass.

RESULTS

The expression of HuR was investigated by immunofluorescence analysis in the adult mouse brain tissue sections. The protein results ubiquitary distributed, and its expression is also present in the SVZ and dentate gyrus areas (figure 1A), the two neurogenic niches in adult mouse brain [19,20].

To investigate the role of HuR in neural differentiation its expression was monitored by immunofluorescence both, in primary adult mouse neural stem cells isolated from the SVZ, and during their differentiation "*in vitro*" [21-23].

NSCs were driven to differentiate through the classical procedure [21], and when cultured in standard medium, with the addition of growth factors (bFGF and EGF) and omission of serum, NCSs form spheroid floating cell aggregates, named neuroshperes. When NSCs are placed into the differentiation induction media, in presence of adhesive molecules (matrigel™), they start to display adhesive ability (figure 1B). The time schedule followed during differentiation has 3 time stones. Day 1, in which neurospheresare mechanically dissociated and single cells are plated in the presence of bFGF (10 ng/ml) and adhesive substrate. At this point, cells start adheres to the plate. Day 3, when cell medium is changed for a serum (fetal bovine serum FBS 2%) containing one, to sustain the differentiation process. Day 6, when cells are differentiated into a mixed neuronal and glial population. To evaluate neural differentiation, cells were

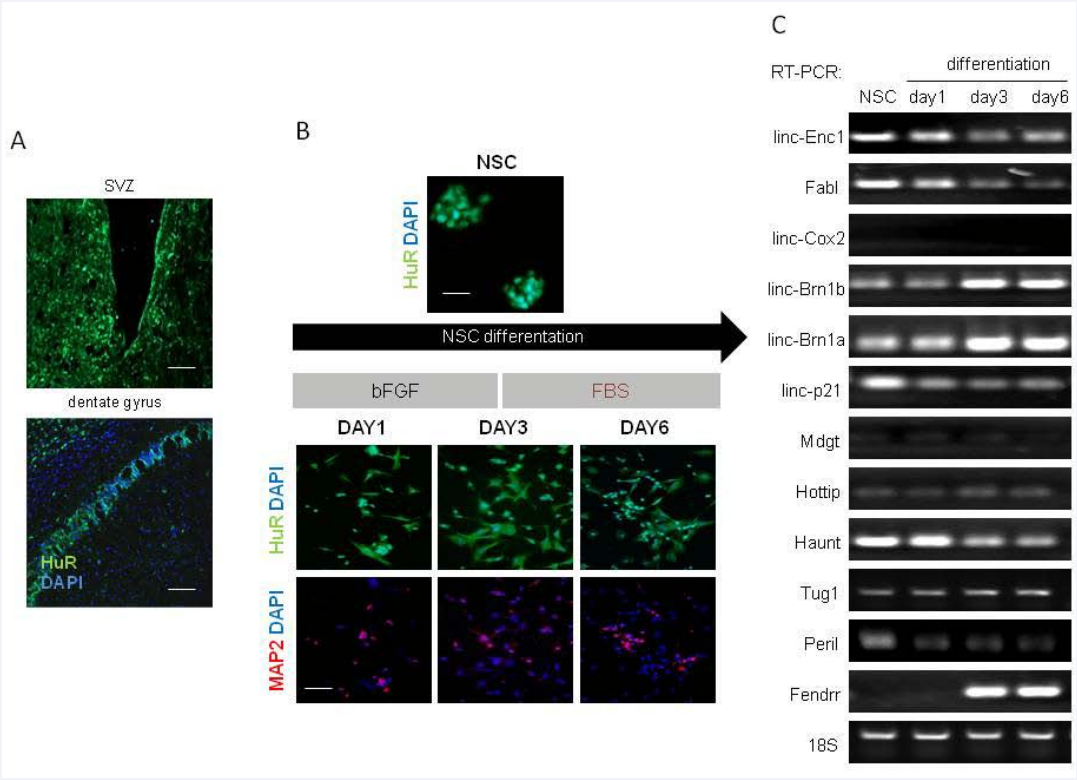


Figure 1 Electrospun nanofibers membrane of poly-ε-caprolactone visualization after 21 days of human Osteoblasts culture (Cells visualization in blue (nucleus /DAPI) and PLL^{FITC} labelled nanofibers in green): colonization and proliferation of osteoblasts into the nanofibers membrane.

monitored for MAP2 neuronal marker expression (Figure 1B) by immunofluorescence.

HuRis expressed in isolated neurospheres and its expression is maintained during each step of the differentiation process (Figure 1B).

Panel C of figure 1 reports the evaluation of lncRNAs expression levels during the NSCs differentiation protocol, investigated by a semi-quantitative RT-PCR. Linc-ENC1, Fabl, linc-p21, Haunt and Peril are down-regulated in the cells when shifting towards the neural fate, instead linc-BRN1a-b and Fendrr show the opposite behavior. LncRNAsHottip, Tug1 andMdgt are not affected by the differentiation process. Linc-COX2 is not expressed and the ribosomal RNA18S was used as loading control (Figure 1C).

HuR is a known RNA-binding-protein studied for its activity in mRNA and lncRNA regulatory activity [17,25]. Here, we tested, by RNA-immuneprecipitation-assay (RIP), for its binding to lincBRN1a, lincBRN1b, lincENC1 and FENDRR both in NSCs, before and after, their differentiation (Figure 2A). The tested lncRNAs present in their sequence several HuR binding sites, as indicated by the prediction tool RBPDB-the database of RNA-binding protein specificities (http://rbpdb.ccbr.utoronto.ca/index.php) (Figure 2B). The protein binds to the investigated lncRNA molecules, when they are expressed, and moreovermitoxantrone (MTX; 2nM), a drug known to inhibit the HuR binding to RNA molecules [18] destroys the interaction between HuR and the various lncRNAs (Figure 2C).

In Figure 2B it is shown the gene expression quantitative RT-PCR, performed both on NSCs before and after their differentiation, in the presence or absence of MTX. LincBRN1a, lincBRN1b and FENDRR results up-regulated during differentiation and MTX treatment is inhibitory. lincENC1 is, on the contrary, down-regulated during neurospheres neural differentiation but, likewise for the other lncRNAs, the blockade of HuR binding prevents such a process (Figure 2C).

In presence of MTX, NSCs maintain a floating spheroid phenotype (Figure 3A) as the cells, even in presence of adhesive substrate, do not adhere to form a classical neural net on the plate. In the presence of MTXneurospheres grow bigger in diameter and higher in number, if compared with the untreated control (Figure 3A). NSCs were then tested for the expression of

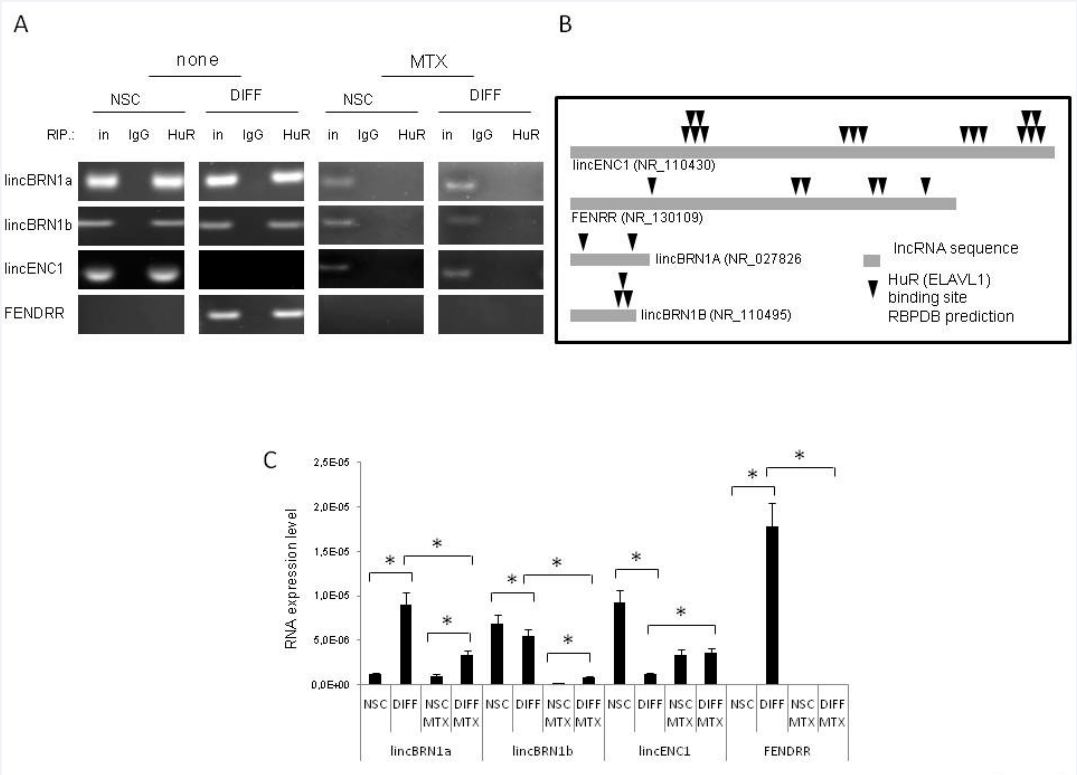


Figure 2 lincRNAs and HuR expression in mouse stem cell niches and during NSCs differentiation *in vitro*. Panel A shows an immunofluorescence to detect HuR (green) in mouse brain at the level of the sub-ventricular zone (SVZ) and dentate gyrus. DAPI (blue) was used as counter-staining. Scale bar 600um.
Panel B shows an immunofluorescence to detect HuR (green) in neurosphers and during the neural differentiation process in vitro. Differentiation takes 6 days (black arrow time-schedule). After dissociation, NSCs are plated on adhesive substrate in culture medium with bFGF (10 ng/ml) for the first 3 days. Then the medium is changed and in the last 3differentiation days the medium issupplemented with serum (FBS 2%) without others growth factors. MAP2 (red) was tested as differentiation quality control and DAPI (blue) was used as counter-staining. Scale bar 100um.
Panel C shows a semi-quantitative RT-PCR on EtBr agarose gel. NSC during "*in vitro*" differentiation (at days 1, 3 and 6) were tested for investigated lncRNAs expression. Linc-ENC1, FABL, HAUNT are remarkably down regulated, instead linc-BRN1a-b, and FENDRR up-regulated. 18S ribosomal RNA is used as loading reference.

nestin, a neural stem cell marker, and beta-tubuilin III (TUJ1), a neuronal precursor marker (Figure 3B). Nestin normally is gradually turned-off as the neuronal differentiation proceeds and on the contrary, TuJ1 expression is increased (Figure 3B; 21,22). Interestingly, MTX treatment inhibits nestin down regulation and maintains TuJ1 expression (Figure 3B).

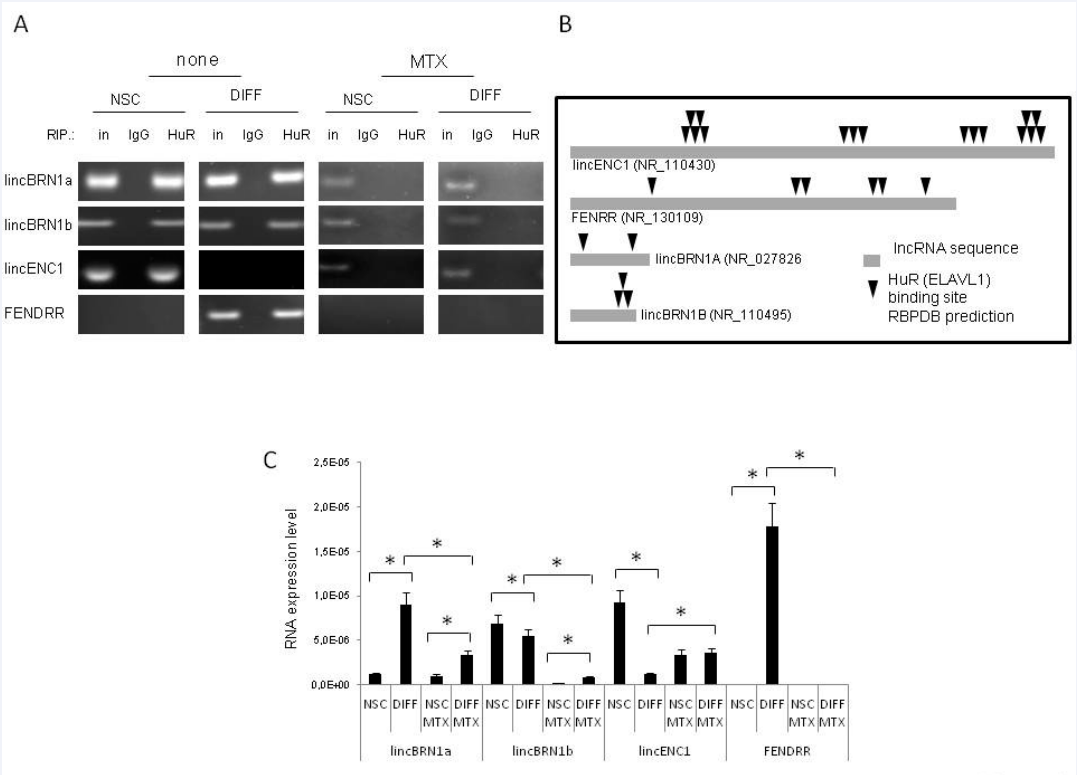
We analyzed in details the expression level of stemness and differentiation markers to understand better the NSCs fate after MTX treatment.

MTX treated neurospheres acquired significantly higher SOX2, NANOG and OCT4 expression level and interestingly in the presence of MTX the NSCs undergoing neural differentiation maintained high level of SOX2, NANOG and OCT4 (Figure 3C). Moreover, the presence of MTX during NSCs differentiation turns-off the expression of the glial marker GFAP (Figure 3C). The neuronal marker TuJ1, normally is expressed by the neurospheres at low level (Figure 3C) [25], but after neural differentiation its induction is up-regulated, and we observed that MTX treatment enhances this up-regulation (Figure 3C). We tested also for the expression level of the astrocytes precursor marker CD44 (26). CD44 resulted expressed in neurospheres and at lower level after differentiation, and we observed that MTX treatment triggers a strong down-regulation of it (Figure 3C). The skin precursor marker Fibronectin (FIBR) was tested as quality control, and in all the experimental conditions resulted negative (Figure 3C) [27].

DISCUSSION

In recent years several publications had reported most of our understanding out of the molecular mechanisms regulating adult neural stem cell specification, proliferation and differentiation (2,3,5). LncRNAs are a quite new class of RNA molecules of which not much is known concerning their molecular way of action, although it seems clear that they are necessary for cell function. In fact, their genetic ablation implies strong cellular and developmental missfunctions, and they play as gene expression regulator but with an unclear molecular mechanism [31]. Recently it has been shown that lncRNAs, are necessary for mouse brain development and neural stem cells differentiation [13].

HuR (Human Antigen R) is a well-characterized protein promoting cell proliferation and involved in RNA splicing and stabilization. The protein is present within the cell in the nuclear



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and cytoplasmic sub-compartments and cooperates with splicing and translation regulatory factors [32-34].

In this paper, we firstly report the ability of HuR to interact with some lncRNAs, already reported to be necessary for mouse brain development and in embryonic stem cells neural differentiation [13]. It is confirmed thatLinc-ENC1, FABL and HAUNT are remarkably down regulated during neural stem cell differentiation, while linc-BRN1a-b, FENDRR are up-regulated. Mitoxantrone (MTX), is an anti-neoplastic and immunesuppressor agent also used in the treatment on multiple sclerosis [35]. Moreover, it has been reported that human pluripotent stem cells are resistant to the *in vitro* treatment with MTX [36]. In neural stem cells, MTX has a strong ability in inducing the up regulation of stemness gene markers (SOX2, OCT4, NANOG) and modulating lncRNAs expression levels during NSCs differentiation towards the levels of expressed as neurospheres.

Recent investigations on HuR network revealed that HuR binds to linc-MD1 and H19 lncRNA. The level of linc-MD1 expression positively correlated with HuR protein abundance, as the level of miR-675, a microRNA embedded in H19 lncRNA [24,37,38]. These authors showed that HuR regulates the fate of linc-MD1 and H19 lncRNA/miR-675, as the cellular depletion of HuR enhances the processing of linc-MD1 into miR-133b and of H19lncRNA into miR-675. HuR role in myogenesis and embryogenesis and its interaction with lncRNAs, regulating their processing to microRNAs, fine-tunes the differentiation program by acting on the microRNA developmental reservoir [25,38]. Our results suggest a new molecular role for the RNA-protein complexes, composed by lncRNAs and HuR, and open a new field of investigation in NSCs biology. In fact, the pharmacological destabilization of these molecular complexes leads to the interesting observation of a NSC failure towards neural differentiation and the enhanced ability in maintaining a strong staminal phenotype. Very recently, HuRwas proposed as a new molecular target for developing agents able to influence its regulating functions on RNA molecules [15].

CONCLUSION

In conclusion, these data encourage us to speculate that the interaction of HuR with specific lncRNAs involved in neural differentiation can be a proper target in the modulation of stemness and differentiation. This opens a new avenue of research that may help the better understanding of the basis underlying neurodegenerative diseases.

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Authors contributions

E.L., A.P., S.C., A.G. designed research;

E.L., T.G., F.C. performed research and analyzed data;

E.L, A.P., S.C., A.D.G. and A.G wrote or contributed to the writing of the paper.

REFERENCES

1. Vishwakarma SK, Bardia A, Tiwari SK, Paspala SA, Khan AA. Current concept in neural regeneration research: NSCs isolation, characterization and transplantation in various neurodegenerative diseases and stroke: A review. J Adv Res. 2014; 5: 277-294.
2. Temple S. Division and differentiation of isolated CNS blast cells in microculture. Nature. 1989; 340: 471-473.
3. Rossi F, Cattaneo E. Opinion: neural stem cell therapy for neurological diseases: dreams and reality. Nat Rev Neurosci. 2002; 3: 401-409.
4. Carletti B, Piemonte F, Rossi F. Neuroprotection: the emerging concept of restorative neural stem cell biology for the treatment of neurodegenerative diseases. Curr Neuropharmacol. 2011; 9: 313-317.
5. Decimo I, Bifari F, Krampera M, Fumagalli G. Neural stem cell niches in health and diseases. Curr Pharm Des. 2012; 18: 1755-1783.
6. Ferraiuolo L, Frakes A, Kaspar BK. Neural stem cells as a therapeutic approach for amyotrophic lateral sclerosis. Mol Ther. 2013; 21: 503-505.
7. Pluchino S, Zanotti L, Deleidi M, Martino G. Neural stem cells and their use as therapeutic tool in neurological disorders. Brain Res Brain Res Rev. 2005; 48: 211-219.
8. Carelli S, Giallongo T, Latorre E, Caremoli F, Gerace C, Basso MD, et al. Adult Mouse Post Mortem Neural Precursors Survive, Differentiate, Counteract Cytokine Production and Promote Functional Recovery after Transplantation in Experimental Traumatic Spinal Cord Injury. J of Stem Cell Res Transplantation. 2014.
9. Carelli S, Giallongo T, Marfia G, Merli D, Ottobrini L, Degrassi A, et al. Exogenous. Adult Post Mortem Neural Precursors attenuate secondary degeneration, and promote myelin sparing and functional recovery following experimental spinal cord injury. Cell Transplant. 2014.
10. Flynn RA, Chang HY. Long noncoding RNAs in cell-fate programming and reprogramming. Cell Stem Cell. 2014; 14: 752-761.
11. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell. 2011; 43: 904-914.
12. Ramos AD, Diaz A, Nellore A, Delgado RN, Park KY, Gonzales-Roybal G, et al. Integration of genome-wide approaches identifies lncRNAs of adult neural stem cells and their progeny in vivo. Cell Stem Cell. 2013; 12: 616-628.
13. Sauvageau M, Goff LA, Lodato S, Bonev B, Groff AF, Gerhardinger C, et al. Multiple knockout mouse models reveal lincRNAs are required for life and brain development. Elife. 2013; 2: e01749.
14. Colombrita C, Silani V, Ratti A. ELAV proteins along evolution: back to the nucleus? Mol Cell Neurosci. 2013; 56: 447-455.
15. Zucal C, D'Agostino V, Loffredo R, Mantelli B, NatthakanThongon, Lal P, et al. Targeting the Multifaceted HuR Protein, Benefits and Caveats. Curr Drug Targets. 2015; 16: 499-515.
16. Wilusz CJ, Wilusz J. HuR and translation--the missing linc(RNA). Mol Cell. 2012; 47: 495-496.
17. Yoon JH, Abdelmohsen K, Srikantan S, Yang X, Martindale JL, De S, et al. LincRNA-p21 suppresses target mRNA translation. Mol Cell. 2012; 47: 648-655.
18. D'Agostino VG, Adami V, Provenzani A. A novel high throughput biochemical assay to evaluate the HuR protein-RNA complex formation. PLoS One. 2013; 8: e72426.
19. Lois C, Alvarez-Buylla A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. Proc Natl Acad Sci U S A. 1993; 90: 2074-2077.

20. Gage FH, Coates PW, Palmer TD, Kuhn HG, Fisher LJ, Suhonen JO, et al. Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc Natl Acad Sci U S A. 1995; 92: 11879-11883.

21. Gritti A, Bonfanti L, Doetsch F, Caille I, Alvarez-Buylla A, Lim DA, et al. Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents. J Neurosci. 2002; 22: 437-445.

22. Marfia G, Madaschi L, Marra F, Menarini M, Bottai D, Formenti A, et al. Adult neural precursors isolated from post mortem brain yield mostly neurons: an erythropoietin-dependent process. Neurobiol Dis. 2011; 43: 86-98.

23. Bottai D, Madaschi L, Di Giulio AM, Gorio A. Viability-dependent promoting action of adult neural precursors in spinal cord injury. Mol Med. 2008; 14: 634-644.

24. Legnini I, Morlando M, Mangiavacchi A, Fatica A, Bozzoni I. A feedforward regulatory loop between HuR and the long noncoding RNA linc-MD1 controls early phases of myogenesis. Mol Cell. 2014; 53: 506-514.

25. Romero-Ramos M, Vourc'h P, Young HE, Lucas PA, Wu Y, Chivatakarn O, et al. Neuronal differentiation of stem cells isolated from adult muscle. J Neurosci Res. 2002; 69: 894-907.

26. Naruse M, Shibasaki K, Yokoyama S, Kurachi M, Ishizaki Y. Dynamic changes of CD44 expression from progenitors to subpopulations of astrocytes and neurons in developing cerebellum. PLoS One. 2013; 8: e53109.

27. Fernandes KJ, McKenzie IA, Mill P, Smith KM, Akhavan M, Barnabé-Heider F, et al. A dermal niche for multipotent adult skin-derived precursor cells. Nat Cell Biol. 2004; 6: 1082-1093.

28. Bottai D, Madaschi L, Di Giulio AM, Gorio A. Viability-dependent promoting action of adult neural precursors in spinal cord injury. Mol Med. 2008; 14: 634-644.

29. Zeng H, Park JW, Guo M, Lin G, Crandall L, Compton T, et al. Lack of ABCG2 expression and side population properties in human pluripotent stem cells. Stem Cells. 2009; 27: 2435-2445.

30. Latorre E, Tebaldi T, Viero G, Spartà AM, Quattrone A, Provenzani A. Downregulation of HuR as a new mechanism of doxorubicin resistance in breast cancer cells. Mol Cancer. 2012; 11: 13.

31. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell. 2013; 152: 1298-1307.

32. Meisner NC1, Filipowicz W. Properties of the Regulatory RNA-Binding Protein HuR and its Role in Controlling miRNA Repression. Adv Exp Med Biol. 2011; 700: 106-123.

33. Yuan Z, Sanders AJ, Ye L, Jiang WG. HuR, a key post-transcriptional regulator, and its implication in progression of breast cancer. Histol Histopathol. 2010; 25: 1331-1340.

34. von Roretz C, Beauchamp P, Di Marco S, Gallouzi IE. HuR and myogenesis: being in the right place at the right time. Biochim Biophys Acta. 2011; 1813: 1663-1667.

35. Martinelli Boneschi F, Vacchi L, Rovaris M, Capra R, Comi G. Mitoxantrone for multiple sclerosis. Cochrane Database Syst Rev. 2013; 5: CD002127

36. Zeng H, Park JW, Guo M, Lin G, Crandall L, Compton T, et al. Lack of ABCG2 expression and side population properties in human pluripotent stem cells. Stem Cells. 2009; 27: 2435-2445.

37. Flynn RA, Chang HY. Long noncoding RNAs in cell-fate programming and reprogramming. Cell Stem Cell. 2014; 14: 752-761.

38. Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G, et al. The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. Nat Cell Biol. 2012; 14: 659-665.

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