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PROFESSIONAL ACCOMPLISHMENTS

EDUCATION

W.A. Engelhardt Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, Russia
Ph.D., Molecular Biology, Chemistry-1986

Moscow Chemical-Technological Institute, Moscow, Russia
MA in Chemistry, Radiochemistry, Biochemistry-1980

EMPLOYMENT HISTORY

Staff Scientist 2004-Present
Professor D. G. Tenen' Laboratory
Beth Israel Deaconess Medical Center, Harvard Institute of Medicine,
Harvard Medical School, Boston, MA

Research Fellow 1999-2004
Assistent Professor R. J. Junghans' Laboratory-Biotherapeutics Development
Beth Israel Deaconess Medical Center, Harvard Institute of Medicine,
Harvard Medical School, Boston, MA

Postdoctoral Assistant 1992-1995
Professor S. Tonegawa' Laboratory-Center for Cancer Research
Massachusetts Institute of Technology, Cambridge, MA

Postdoctoral Fellow 1991-1992
Professor J. Sambrook' Laboratory-Department of Biochemistry, University of Texas
Southwestern Medical Center at Dallas, Dallas, TX

Senior Investigator 1990-1991
Institute of Gene Biology, Moscow
Academy of Sciences of the USSR, Moscow, Russia

Visiting Lecturer 1989-1990
Department of Tumor Biology, Institute for Cancer Research
The Norwegian Radium Hospital, Oslo, Norway

Researcher 1980-1989
Professor G.P. Georgiev' Laboratory, W.A. Engelhardt Institute of Molecular Biology
Academy of Sciences of the USSR, Moscow, Russia

Contributions to RNA Science

1. **Discovered a novel RNA-mediated mechanism for the establishment of epigenetic marks and cell-specific epigenetic profiles, thereby providing a unifying explanation for the accuracy and persistence of epigenetic marks on chromatin.**
   a. **Alexander K. Ebralidze**, Simone Ummarino, Mahmoud A. Bassal, Haoran Zhang, Bogdan Budnik, Emanuele Monteleone, Dennis Kappei, Yanjing V. Liu, Danielle E.Tenen, Rory Coffey, Mee Rie Sheen, Yanzhou Zhang, Anaïs Wanet, Bon Q. Trinh, Valeria Poli, Vladimir Espinosa Angarica, Roberto Tirado Magallanes, Touati Benoukraf, Colyn CraneRobinson, Annalisa Di Ruscio, and Daniel G. Tenen. Formation and recycling of an active epigenetic mark mediated by cell cycle-specific RNAs. Formation and recycling of an active epigenetic mark mediated by cell cycle-specific RNAs. DOI:10.21203/rs.3.rs-464637/v1. Under revision in *Nature Genetics*.

2. **Discovered a novel class of RNAs, the DNMT1-interacting RNAs (DiRs), play a key role in the control of cell type-specific DNA methylation patterns.**
   b. Di Ruscio A*, **Ebralidze AK***, Benoukraf T, Amabile G, Goff LA, Terragni J, Figueroa ME,De Figueiredo Pontes LL, Alberich-Jorda M, Zhang P, Wu M, D'Alo F, Melnick A, Leone G, Ebralidze KK, Pradhan S, Rinn JL, Tenen DG. DNMT1-interacting RNAs block gene-specific DNA methylation. *Nature* 503:371-376, 2013. PMCID 3870304 *- equal contribution.
   b. Amabile G, Di Ruscio A, Müller F, Welner RS, Yang H, **Ebralidze AK**, Zhang H, Levantini E Qi L, Martinelli G, Brummelkamp T, Le Beau MM, Figueroa ME, Bock C, Tenen DG., Dissecting the role of aberrant DNA methylation in human leukaemia. *Nat Commun*. 2015 May 22;6:7091. doi: 10.1038/ncomms8091. PMC4443494

3. **Identified molecular cause of the Myotonic Dystrophy (DM) pathogenesis - "transcription factor syndrome" (also applicable for other Trinucleotide Repeat Disorders)**
   a. **Ebralidze A**, Y. Wang, V. Petkova, K. Ebralidse, and R. P. Junghans. RNA leaching of transcription factors disrupts transcription in Myotonic Dystrophy. *Science* 2004, 303, 383-7. PMID: 14657503
   b. Junghans RP, **Ebralidze A**, Tiwari B. Does (CUG)n repeat in DMPK mRNA 'paint' chromosome 19 to suppress distant genes to create the diverse phenotype of myotonic dystrophy? A new hypothessis of long-range cis auotosomal inactivation. *Neurogenetics* 2001, 3:59-67. PMID: 11354827

4. **Identified phylogenetically conserved elements involved in the initiation of antisense transcription and that these antisense RNAs function as important modulators of proper dosages of PU.1 gene**.
   a. Hoogenkamp M, Krysinska H, Ingram R, Huang G, Barlow R, Clarke D, **Ebralidze A**, Zhang P, Tagoh H, Cockerill PN, Tenen DG, Bonifer C. The Pu.1 locus is differentially regulated at the level of chromatin structure and noncoding transcription by alternate mechanisms at distinct developmental stages of hematopoiesis. *Mol Cell Biol*. 2007 Nov;27(21):7425-38. PMCID: PMC2169062
   b. **Ebralidze A**. et al. PU.1 expression is modulated by the balance of functional sense and antisense RNAs. *Genes and Development*. 2008; 22: 2085-2092. PMC2492744

Personal Statement

I received my PhD degree from W.A. Engelhardt Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, Russia. I joined Prof. D.G. Tenen Laboratory at Beth Israel Deaconess Medical Center/Harvard Medical School as Junior Faculty: Instructor in Medicine in 2004. I was promoted to Principal Associate in Medicine in 2018. My research is focused on chromatin architecture, transcription factors, non-coding RNAs and their function in gene regulation and genomic replication during normal and aberrant cell development, understanding how these factors become deregulated in their function in diseases.
Currently I am working on the uncovering the roles of the noncoding RNAs in the establishment of the epigenetic patterns. We have generated the data supporting that epigenetic modification is triggered by locus-specific genetic events. We prove that the appearance of the major epigenetic mark-histone modification-acetylated histone H2A.Z is regulated by the encoded within the respective gene loci long noncoding RNAs. These transcripts, termed Spears (S Phase EArly RNAs) are induced in the early S phase in the vicinity of the corresponding gene transcription start site. We show that Spears through forming ribonucleoprotein complexes with the histone H2A.Z and histone acetyl transferase Tip60 recruit acetylated form of the histone

variant H2A.Z. The interaction of the lncRNAs with the activation mark provides a potentially unifying mechanistic explanation for the accuracy and persistence of the epigenetic marks. Our research team has already finished the experimental part of the investigations and manuscript was submitted for publication in *Nature Genetics*; currently we are revising the manuscript according to the reviewer's comments (Preprint: Ebralidze et al., DOI:10.21203/rs.3.rs-464637/v1.). These recent studies have made me take a new look at the problem of Myotonic Dystrophy (DM1) pathogenesis *Science* 2004, 303, 383-7. PMID: 14657503; Neurogenetics 2001 Mar;3(2):59-67. doi: 10.1007/s100480000103), as well as other Trinucleotide Repeat Disorders that are caused by expansion of trinucleotide repeats (CAG, CTG, CGG, and GAA). I am developing a program aiming at directly reversing the pathological expansion of the repeat size to its normal baseline.

Peer Review Publications:
https://pubmed.ncbi.nlm.nih.gov/?term=Ebralidze%20AK

Selected Peer Review Publications (chronological order):

1. Tchurikov NA, **Ebralidze AK**, Georgiev GP. The suffix sequence is involved in processing the 3' ends of different mRNAs in Drosophila melanogaster. **EMBO J**. 1986 Sep;5(9):2341-2347.
2. Churikov NA, **Ebralidze AK**, Polukarova LG (1987). Cut locus of Drosophila melanogaster: The suffix sequence found in the locus is involved in the 3'-end maturation of different Drosophila mRNAs. **Genetika** 23(10):1807-22.
3. **Ebralidze AK**, Tulchinsky EM, Grigorian MS, Senin VM, Lukanidin EM (1989). Isolation and characterization of a gene specifically expressed in different metastatic cells and whose deduced gene product has a high degree of homology to a Ca2+-binding protein family. **Genes Dev**. 3(7):1086-93.
4. **Ebralidze AK**, Tulchinsky EM, Grigorian MS, Senin VM, Lukanidin EM (1989). Isolation of cDNA clones which are specifically transcribed in metastatic versus nonmetatstatic murine tumors. **Genetika** 25(5):932-6.
5. Grigorian MS, **Ebralidze AK**, Tulchinsky EM, Revazova ES, Senin ES, Lukanidin EM (1989). Transcription of the DNA of mts271 clone in various tumor and normal mouse cells. **Genetika** 25(6):993-1000.
6. Tulchinskii EM, **Ebralidze AK**, Grigorian MS, Milschina NV, Lukanidin EM (1989). Structure of the mts271 gene, encoding a new calcium-binding protein. **Genetika** 25(7):1150-9.
7. Tulchinsky EM, Grigorian MS, **Ebralidze AK**, Mislhina NI, Lukanidin EM (1990). Structure of gene mts1, transcribed in metastatic mouse tumor cells. **Gene** 87(2):219-23.
8. Cardenas MA, Vorovich MF, **Ebralidze AK**, Chertov Oiu, Lukanidin EM (1993). Prepapration of recombinant metastazin and charasterisitics of it. **Bioorg Chem** 19(4):420-6.
9. Grigorian MS, Tulchinsky EM, Zain S, **Ebralidze AK**, Kramerov DA, Krajevska MV, Georgiev GP, Lukanidin EM (1993). The mts1 gene and control of tumor metastatsis. **Gene** 135(1-2):229-38.
10. Ji Z, **Ebralidze A**, Tonegawa S, and Vogel MW (1996). Spinocerebellar mossy fiber terminal topography in the NMDAR2/PKC double mutant cerebellum. **Brain Res Dev Brain Res** 97(1):13842.
11. Poss KD, Thomas MJ, Ebralidze AK, O'Dell TJ, Tonegawa S (1995). Hippocampal long-term potentiation is normal in heme oxygenase-2 mutant mice. **Neuron** 15(4):867-73.
12. **Ebralidze AK**, Rossi DJ, Tonegawa S, Slater NT (1996). Modification of NMDA receptor channels and synaptic transmission by targeted disruption of the NR2C gene. J **Neuroscience** 16 (16):5014-25.
13. Junghans RP, **Ebralidze A**, Tiwari B. (2001). Does (CUG)n repeat in DMPK mRNA 'paint' chromosome 19 to supress distant genes to create the diverse phenotype of myotonic dystrophy? A new hypothesis of long-range cis autosomal inactivations. **Neurogenetics** 3:59-67.
14. **A. Ebralidze**, Y. Wang, V. Petkova, K. Ebralidse, and R. P. Junghans (2004). RNA leaching of transcription factors disrupts transcription in Myotonic Dystrophy. **Science** 303, 383-7.
15. Steidl, U., Rosenbauer, F., Verhaak, R.G., Gu, X., **Ebralidze, A**., Otu, H.H., Klippel, S., Steidl, C., Bruns, I., Costa, D.B., et al. (2006). Essential role of Jun family transcription factors in PU.1 knockdown-induced leukemic stem cells. **Nat Genet** 38, 1269-1277.
16. Steidl U, Steidl C, **Ebralidze A**, Han HJ, Rosenbauer F, Koschmieder S, Wagner K, Kobayashi S, Schulz T, Becker A, O'Brien KB, Krauter J, Haase D, Verhaak RGW, Delwel R, Truemper L, Kohwi-Shigematsu T, Griesinger F, Tenen DG. A distal single nucleotide polymorphism alters long-range regulation of the PU.1 gene in acute myeloid leukemia. **J Clin Invest**. 117(9):2611-2620 (2007).
17. Hoogenkamp M, Krysinska H, Ingram R, Huang G, Barlow R, Clarke D, **Ebralidze A**, Zhang P, Tagoh H, Cockerill PN, Tenen DG, Bonifer C. The Pu.1 locus is differentially regulated at the level of chromatin structure and non-coding transcription by alternate mechanisms at distinct developmental stages of hematopoiesis. **Mol Cell Biol**. 2007 Sep 4; [Epub ahead of print].
18. **Ebralidze A**; F.C. Guibal, U. Steidl, P. Zhang, S. Lee, B. Bartholdy, V. Petkova, F. Rosenbauer, G. Huang, T. Dayaram, J. Klupp, K. O'Brien, B. Will, M. Hoogenkamp, C. Bonifer, D. G. Tenen. Biogenesis of sense and functional antisense gene RNAs through shared chromatin architecture. PU.1 expression is modulated by the balance of functional sense and

antisense RNAs. **Genes Dev**. 2008; 22: 2085-2092.

19. Guibal FC, Alberich-Jorda M, Hirai H, **Ebralidze A**, Levantini E, Di Ruscio A, Zhang P, Santana-Lemos BA, Neuberg D, Wagers AJ, Rego EM, Tenen DG. Identification of a myeloid committed progenitor as the cancer initiating cell in acute promyelocytic leukemia. **Blood**. 2009 Dec; 114: 5415-25. PMID: 19797526 [PubMed - indexed for MEDLINE].

20. O'Brien, Alberich-Jordà, Yadav, Kocher, DiRuscio, **Ebralidze A**, Levantini, Sng, Bhasin, Caron, Kim, Steidl, Huang, Halmos, Rodig, Bedford, Tenen, and Kobayashi. CARM1 is required for proper control of proliferation and differentiation of pulmonary epithelial cells. **Development** 2010 Jul;137(13):2147-56. PMID: 20530543 [PubMed - indexed for MEDLINE].

21. Levantini E, Lee S, Radomska HS, Hetherington CJ, Alberich-Jorda M, Amabile G, Zhang P, Gonzalez DA, Zhang J, Basseres DS, Wilson NK, Koschmieder S, Huang G, Zhang DE, **Ebralidze AK**, Bonifer C, Okuno Y, Gottgens B, Tenen DG. RUNX1 regulates the CD34 gene in haematopoietic stem cells by mediating interactions with a distal regulatory element. **EMBO J**. 2011 Aug 26;30(19):4059-70. PMID: 21873977.

22. Alberich-Jorda M, Wouters B, Balastik M, Shapiro-Koss C, Zhang H, Di Ruscio A, Radomska HS, **Ebralidze AK**, Amabile G, Ye M, Zhang J, Lowers I, Avellino R, Melnick A, Figueroa ME, Valk PJ, Delwel R, Tenen DG. C/EBPgamma deregulation results in differentiation arrest in acute myeloid leukemia. **J Clin Invest** 122:4490-4504, 2012. PMCID PMCID 3533560.

23. Di Ruscio A, **Ebralidze AK**, Benoukraf T, Amabile G, Goff LA, Terragni J, Figueroa ME, De Figueiredo Pontes LL, Alberich-Jorda M, Zhang P, Wu M, D'Alo F, Melnick A, Leone G, Ebralidze KK, Pradhan S, Rinn JL, Tenen DG. DNMT1-interacting RNAs block gene-specific DNA methylation. **Nature** 503:371-376, 2013. PMCID 3870304.

24. Amabile G, Welner RS, Nombela-Arrieta C, D'Alise AM, Di Ruscio A, **Ebralidze AK**, Kraytsberg Y, Ye M, Kocher O, Neuberg DS, Khrapko K, Silberstein LE, Tenen DG. In vivo generation of transplantable human hematopoietic cells from induced pluripotent stem cells. **Blood**. 2012 Dec 3;122(12):4490-504. PMID: 23212524 [PubMed - indexed for MEDLINE].

25. Zhang H, Alberich-Jorda M, Amabile G, Yang H, Staber PB, Di Ruscio A, Welner RS, **Ebralidze A**, Zhang J, Levantini E, Lefebvre V, Valk PJ, Delwel R, Hoogenkamp M, Nerlov C, Cammenga J, Saez B, Scadden DT, Bonifer C, Ye M, Tenen DG. Sox4 Is a Key Oncogenic Target in C/EBPα Mutant Acute Myeloid Leukemia. **Cancer Cell**. 2013 Nov 11;24(5):575-88. PMID: 24183681 [PubMed - indexed for MEDLINE].

26. Staber PB, Zhang P, Ye M, Welner RS, Levantini E, Di Ruscio A, **Ebralidze AK**, Bach C, Zhang H, Zhang J, Vanura K, Delwel R, Yang H, Huang G, Tenen DG. The Runx-PU.1 pathway preserves normal and AML/ETO9a leukemic stem cells. **Blood**. 2014 Sep 3. pii: blood-2014-01-550855. [Epub ahead of print]

27. Amabile G, Di Ruscio A, Müller F, Welner RS, Yang H, **Ebralidze AK**, Zhang H, Levantini E, Qi L, Martinelli G, Brummelkamp T, Le Beau MM, Figueroa ME, Bock C, Tenen DG. Dissecting the role of aberrant DNA methylation in human leukaemia. **Nat Commun**. 2015 May 22;6:7091. doi: 10.1038/ncomms8091. PMID:25997600.

28. Bararia D, Kwok HS, Welner RS, Numata A, Sárosi MB, Yang H, Wee S, Tschuri S, Ray D, Weigert O, Levantini E, **Ebralidze AK**, Gunaratne J, Tenen DG. Acetylation of C/EBPα inhibits its granulopoietic function. **Nat Commun**. 2016 Mar 23;7:10968. doi: 10.1038/ncomms10968. PMID: 27005833.

29. Gonzalez D, Luyten A, Bartholdy B, Zhou Q, Kardosova M, **Ebralidze A**, Swanson KD, Radomska H, Zhang P, Kobayashi SS, Welner RS, Levantini E, Steidl U, Chong G, Collombet S, Choi MH, Friedman AD, Scott LM, Alberich-Jorda M, Tenen DG. ZNF143 is an important regulator of the myeloid transcription factor C/EBPα**. J Biol Chem**. 2017 Sep 12. pii: jbc.M117.811109. doi: 10.1074/jbc.M117.811109. [Epub ahead of print].

30. Canesin G, Di Ruscio A, Li M, Ummarino S, Hedblom A, Choudhury R, Krzyzanowska A, Csizmadia E, Palominos M, Stiehm A, **Ebralidze A**, Chen SY, Bassal MA, Zhao P, Tolosano E, Hurley L, Bjartell A, Tenen DG, Wegiel B. Scavenging of Labile Heme by Hemopexin Is a Key Checkpoint in Cancer Growth and Metastases. **Cell Rep**. 2020 Sep 22;32(12):108181. doi: 10.1016/j.celrep.2020.108181. PMID: 32966797.

31. van der Kouwe E, Heller G, Czibere A, Pulikkan JA, Agreiter C, Castilla LH, Delwel R, Di Ruscio A, **Ebralidze AK**, Forte M, Grebien F, Heyes E, Kazianka L, Klinger J, Kornauth C, Le T, Lind K, Barbosa IAM, Pemovska T, Pichler AS, Schmolke AS, Schweicker CM, Sill H, Sperr WR, Spittler A, Surapally S, Trinh BQ, Valent P, Vanura K, Welner RS, Zuber J, Tenen DG, Staber PB. Core binding factor leukemia hijacks T-cell prone PU.1 antisense promoter. **Blood**. 2021 May 19:blood.2020008971. doi: 10.1182/blood.2020008971. Online ahead of print. PMID: 34010414

32. Trinh B, Ummarino S, Zhang Y, **Ebralidze AK**, Bassal M, Nguyen T, Heller G, Coffey R, Tenen D, van der Kouwe E, Fabiani E, Gurnari C, Wu C-H, Espinosa Angarica V, Yang H, Chen S, Zhang H, Thurm A, Marchi F, Levantini E, Staber P, Zhang P, Voso M, Pandolfi PP, Kobayashi S, Chai L, Di Ruscio A, Tenen DG. Myeloid lncRNA LOUP Mediates Opposing Regulatory Effects of RUNX1 and RUNX1-ETO in t(8;21) AML. **Blood**. 2021 May 10;blood.2020007920. doi: 10.1182/blood.2020007920. Online ahead of print. PMID: 33971010.

Letter of Application_Alexander K. Ebralidze

This proposal is being submitted in response to The RNA Society Outstanding Career Researcher Award.

I am working closely with my Unit Director, Daniel G. Tenen, at Beth Israel Deaconess Medical Center, Harvard Medical School, Boston USA

As the Research Specialist, I will be focused on project: “Long noncoding RNA regulation of transcription and epigenetic memory”.

The ***scientific premise*** for this proposed study is built on our publications investigating the roles of different classes of functional RNAs: architectural antisense RNAs (AS) (Genes Dev. 22:2085, 2008); genomic methylation-regulating noncoding RNAs (DiRs) (Nature 503:371, 2013); RNAs involved in formation and recycling of an active epigenetic mark (SPEARs) (Nature Genet. under revision, pre-print: 10.21203/rs.3.rs-464637/v1); and mutant RNAs transcribed from the deletirious repeat expansion (Science 303:383, 2004). Understanding the role of lncRNAs in the formation of transcriptionally active or silent chromatin through a novel mechanism for the biosynthesis and maintenance of a major epigenetic mark and inheritance of site-specific epigenetic states. We will show that these cell cycle-specific RNAs are conduits of the accurate recycling of the epigenetic mark and thereby provide transgenerational regulation of the transcriptional activity of the respective genomic loci. This discovery will resolve the long-standing dispute between supporters of epigenetics and genetics, allowing the two camps to change the way they perceive chromatin modifications and the very definition of epigenetic memory. Recently we have shown: a) that the formation of the active epigenetic marks is regulated by a novel class of cell cycle-specific RNAs, which we named SPEARs (S Phase EArly RNAs); and b) SPEARs play a fundamental role in the transmission and propagation of epigenetic states through cell divisions. (Nature Genet. under revision, pre-print: 10.21203/rs.3.rs-464637/v1).

We ***hypothesize*** that acetylated H2A.Z (acH2A.Z) is not the only epigenetic mark which is regulated by long ncRNAs. In this study we propose to investigate mechanism(s) of the biosynthesis and maintenance of another major epigenetic marks: replacement histone H3.3 (the major H3 variant). Once completed, this study will redefine the concept of epigenetic memory, which will help resolve long-standing discussions about the relationship between genetics and epigenetics, allowing the two camps to change the way they perceive chromatin modifications and the very definition of epigenetic memory. Importantly, the study will pave the way for the uncovering how epigenetic information is encoded and regulated under normal conditions and in disease states.

I propose: **(1a). Identification of “promoter” RNAs induced at the different time-points of the S phase.** By using “Click” technology we will capture and sequence nascent RNAs encoded in the regions adjacent to the promoters of the transcribed genes and induced in the different segments of the S phase – RNAs will be collected at 0, 2, 4, 6 and 8hr after release of synchronized human HL-60 cells into S phase. We will utilize Nanopore sequencing, which enables direct, real-time analysis of long DNA and RNA species. **(1b). Identification of induced “promoter” RNAs that form ribonucleoprotein complexes (RNPs) with the replacement histone H3.3 and its acetylated forms at different time-points of S phase.** We will male use of RNA and chromatin immunoprecipitation (RIP-seq and ChIP-seq) together with Mass Spectrometry. The results of H3.3/acH3.3-RIP-seq will be verified by “reverse” protocols: RNA-pull-down followed by mass spectrometry analyses of the precipitated proteins. Only the RNA species forming RNPs in both approaches will be taken for further analyses. **(1c). Correlation of the expressed promoter RNAs with that of neighboring genes and levels of deposition of H3.3/acH3.3 at the mRNA transcription start sites (TSSs).** (i) *Loss-of-function*. We will use two transcription inhibitors, Actinomycin D and DRB. In addition to this global pharmacological approach we will use targeted RNAi and CRISPR-based platforms. Decreasing levels of targeted RNA expression will be compared to the decrease of RNA from the linked gene and the levels of H3.3/acH3.3 deposition assessed from nascent ChIP-PCR/ChIP-seq (ii) *Gain-of-function*. We will use the dCas9-VP64 gene activation system. Increasing levels of targeted RNA expression will be compared to the increase of the linked gene and the level of H3.3/acH3.3 deposition, using nascent ChIP-PCR/ChIP-seq. **(1d). Correlation of the expression of the identified promoter RNAs with the recycling of the epigenetic mark - H3.3/acH3.3.** To evaluate the role of the identified promoter RNAs in the recycling of the H3.3/acH3.3 mark, we will perform the SILAC experiments. This experiments will enable investigation of whether “old” histones with “old” modifications are preserved and transmitted through the generational border. ***Additional and/or Future Directions:*** This aims to focus on the deposition/recycling of H3.3/acH3.3 and H2A.Z within the loci of tumor suppressors *PU.1*, *CDKN2A/B* and c-MYC proto-oncogene.

October 31, 2021

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Re: Letter of Support from the Unit Director _ Alex Ebraldize_ The RNA Society Outstanding Career Researcher Award

Dear Members of the Review Committee:

I give my strongest support for Dr. Ebralidze's application for this award. By strongest support, I mean the strongest letter of support that I have ever written in the 37 years of running an independent laboratory. In brief, Dr. Ebralidze is the most brilliant investigator I have worked with, who continually comes up with groundbreaking discoveries.

His original discoveries and publications were a major factor towards my receiving a R35 Outstanding Investigator Award, and his continued efforts and discoveries will form the basis of my renewal application.

Dr. Ebralidze comes from the highest quality of training background: Ph.D. in biochemistry and molecular biology in Moscow (they know real biochemistry there!); postdoctoral training with Joe Sambrook (one of the pioneers in the molecular biology of cancer), and Susumu Tonegawa (Nobel Laureate in Medicine). Since 1999 he has been at our institution, and every 4-5 years publishes a landmark paper related to the general theme of RNA, gene regulation, chromatin, and now cancer. What is remarkable is that in each case, it was Dr. Ebralidze's brilliant ideas and experimental skills which led to these discoveries, not those of his supervisor (I know this first-hand!). At the same time, he has been a leader for people in my group and other labs, both by example, and also by direct teaching. In spite of his brilliance, he is a very patient, kind individual, and a great teacher.

Dr. Ebralidze has pioneered a number of groundbreaking studies, and like fine wine, they just get better and better. While in Dick Junghans' lab, he hypothesized and then demonstrated a novel mechanism, in which a mutant RNA sequestered a transcription factor, published as a first author paper in Science. This was a result of Dr. Ebralidze's original ideas, as Dick (Junghans) is an accomplished investigator in other areas of research.

Dr. Ebralidze joined my group in 2004. Since then, he has iled studies which changed the direction of my laboratory, from studying transcription factors in a traditional manner to the role of long ncRNAs in cancer. Studies that he originated led to two thirds of the ideas in my R35 Outstanding Investigator application in 2014, and will lead to three quarters of my R35 renewal application. His first author publication in Genes and Development in 2008 led to two back-to-back publications with accompanying editorial recently (October 14, 2021) in the same issue of Blood. His 2013 first author Nature paper (he kindly shared first authorship with a then fairly inexperienced but talented hematologist) led to development of a novel gene locus specific demethylation strategy, which led to a number of published studies, both from our group and validated by a number of independent studies from other laboratories. Even more exciting are additional studies from this work under review or about to be submitted, including the first example of demethylation therapy leading to activating an oncogene leading to poor survival of patients in remission, currently under revision for the New England Journal of Medicine, and another study identifying a novel genome wide demethylation signature identifying an important control element important for establishment of promoter-enhancer interactions and activation of transcription of hundreds of mRNAs.

Perhaps most exciting are his recent discoveries leading to major novel findings of classes of long noncoding RNAs which activate downstream mRNA expression through modification of a variant histone and also lead to epigenetic "memory" of this modification through cell division; and another class which regulate mammalian origins of DNA replication. In addition, he has strong preliminary data on the role of noncoding RNAs in maintaining genomic stability in normal and leukemic cells, which of course is highly relevant to the mission of RNA Society.

There is a common theme in his work: original ideas, brilliant execution of experimentally challenging studies,

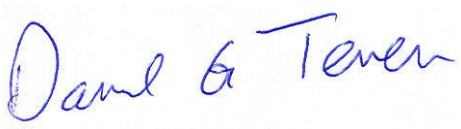
and novel discoveries which lead to potentially novel therapeutic modalities in cancer.

In addition to his own studies, Dr. Ebralidze is a great asset to other members of my group, other laboratories, and to the Institute for RNA Medicine at the Beth Israel Deaconess Cancer Center. As an example, he transformed a clinical hematologist (Annalisa Di Ruscio) into a hard core RNA biochemist and molecular biologist, and generously insisted that she be “first” first author of their 2013 Nature paper. In many respects, Dr. Ebralidze served as first and last author of that work, but he unselfishly wanted to promote her career as a young investigator.

I cannot think of a better candidate for this award, which is designed to give individuals like him the well-deserved recognition.

In summary, I support this application with my strongest recommendation that I have ever given to Dr. Ebralidze, a brilliant scientist for whom this award seems to be precisely designed. Receiving this award will ensure that he continues to produce great discoveries with potential therapeutic implications in cancer.

Sincerely,



Daniel G. Tenen, M.D.
Professor of Medicine, Harvard Medical School
Blood Program Leader, Harvard Stem Cell Institute
Member, Institute for RNA Medicine, Beth Israel Deaconess Cancer Center