

CV Antonello (Antonio) Mallamaci, July 2020

A. Generalities and present position

Generalities: Antonello (Antonio) Mallamaci, born on June 14, 1961, in Naples, Italy, Italian citizen
 Present position: Full Professor of Molecular Biology, at SISSA, Trieste, Italy

B. Education

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Liceo-Ginnasio A.Pansini, Naples, Italy	Diploma	1979	High School, Humanities branch
Conservatorio di Musica S. Pietro a Majella, Naples, Italy	Diploma	1985	Piano
University of Naples Federico II, Italy	Biol. Sci. Degree	1990	Biological Sciences
MPI fur Biophysikalische Chemie, Goettingen, Germany	Course Attend.	1995	Mouse and Chick Development
CSHL, Cold Spring Harbor, USA	Course Attend.	1996	Mouse Development

C. Positions

Positions and Employment

- 1993 Fellow, National Institute for Medical Research, Medical Research Council, London, UK;
 1993-1997 Fellow, Department of Biological and Technological Research, Scientific Institute H San Raffaele, Milan, Italy
 1997 Fellow, GSF, Forschungszentrum fur Umwelt und Gesundheit, Munich, Germany
 1997-2001 Tenured Researcher, Department of Biological and Tecnological Research - Scientific Institute H San Raffaele, Milan, Italy
 2002-2006 Head of Laboratory, Department of Biological and Tecnological Research - Scientific Institute H San Raffaele, Milan, Italy
 2006-2018 Head of Laboratory, Associate Professor of Molecular Biology
 from 2014 to 2018, Coordinator of the PhD Course in Functional and Structural Genomics
 at Scuola Internazionale Superiore di Studi Avanzati, SISSA, Trieste, Italy
 2018-2020 Head of Laboratory, Full Professor of Molecular Biology
 since 2018, Coordinator of the Neuroscience Area
 at Scuola Internazionale Superiore di Studi Avanzati, SISSA, Trieste, Italy

Other Experience

- 1997-1999 Appointed Professor ("Developmental Biology and Vegetal Morphogenesis"), University of Verona, Verona, Italy
 1999-2002 Appointed Professor ("Developmental Genetics: from Drosophila to Vertebrates"), University of Pavia, Pavia, Italy
 2002-2003 Appointed Professor ("Recombinant DNA technologies"), University of Insubria, Varese, Italy;
 2003-2005 Appointed Professor ("Animal models in Genetics"), University Vita-Salute San Raffaele, Milan, Italy;
 2000-2005 Courses Organizer and Teacher, Open University Ph.D. Program in Cell and Molecular Biology, Department of Biological and Tecnological Research, Scientific Institute H San Raffaele & University Vita-Salute San Raffaele, Milan, Italy
 2007-2020 Appointed Professor ("CNS development", 2007; "Cerebral Cortex Development", 2008-2009; "Neuroembryology", 2009-2013; "Neurodevelopmental genetics", 2014-present), University of Trieste, Trieste, Italy

- 2002-2020 Ad hoc reviewer for the Journals: Brain Research - BMC Biology - BMC Genomics - BMC Neural Development - Cell Cycle - Cellular and Molecular Life Sciences - Cerebral Cortex - Development - Developmental Biology - Developmental Dynamics - European Journal of Neuroscience - EMBO Journal - Experimental Neurology - FEBS Letters - Frontiers in Neuroscience - Gene - ISRN Stem Cells - Journal of Comparative Neurology - Journal of Biomedical Research - Journal of Neuroscience - Mechanisms of Development - Molecular Cancer Therapy - Neural Development - Neurochemical Research - Neuroscience - PLoS Genetics - Stem Cell
- 2002-2020 Ad hoc reviewer for the Funding Agencies: ANR/ Agence Nationale de la Recherche - Association for International Cancer Research - Israel Science Foundation - The Wellcome Trust - IASys/ India Alliance System - FWF/Austrian Science Fund - University of Rome - University of Trieste.

D. Contribution to Science

A small set of evolutionarily conserved transcription factor genes specifies the early cerebral cortical field and subsequently dictates its inter-areal differentiation.

At the end of last century, molecular mechanisms orchestrating early patterning of the rostral mammalian brain were still largely unknown. The expression profiles of a number of genes encoding for secreted ligands and transcription factor genes suspected to be involved in this process were known. However the causative role of these genes in CNS patterning was still hypothetical. First as senior researcher (corresponding author) and then as PI, I substantially contributed to decode gene control of this process. My Team demonstrated that *Emx2* and *Pax6* are each sufficient to specify pallial identity, so that mice null for both genes undergo homeotic respecification of dorsal telencephalic territories to striatal identity. Moreover, we showed that, as the pallial field is specified, then *Emx2* (as well as its paralog *Emx1*), *Pax6* and *Foxg1* shape its areal profile. Specifically, we found that *Emx2* promotes hippocampal and occipital neocortical programs, *Pax6* is needed for proper development of fronto-lateral areas, *Foxg1* is absolutely required for the activation of paleocortical and neocortical programs. Moreover, we found that *Emx2* impact on cortical arealization has a dual origin. It stems from the capability of this gene to allot a proper number of pallial precursors to caudal-medial programs. It reflects its ability to subsequently sustain the expansion of such committed caudal-medial proliferating pool.

Control of late neocortical histogenesis by transcription factor genes patterning the pallial field.

Beyond their involvement in early brain patterning and their fine control of neuronogenesis dynamics, *Emx2* and *Foxg1* transcription factor genes were known to be expressed in late embryonic neocortex, however their role in this developmental scenario was for a long time largely obscure.

My Team investigated the impact of *Foxg1* on the architecture of neocortical pyramidal neurons and found that it promotes dendrite elongation and branching. That was early reported on Stem Cells in 2010. A more in depth study on this issue, including a detailed reconstruction of underlying molecular mechanisms, was subsequently run. Its results were published in Cerebral Cortex in 2018.

Next, we investigated the impact of *Foxg1* on the activity of neocortical pyramidal neurons. We found that mice overexpressing *Foxg1* displayed an EEG with increased spike frequency and were more prone to KA-induced seizures. Consistently, primary cultures of neocortical neurons overexpressing *Foxg1* were hyperactive, which reflected an unbalanced expression of key determinants of neuron excitability as well as by a pronounced interneuron depletion. We also detected a transient *Foxg1* upregulation driven by neuronal activity and reconstructed the underlying gene circuitry. Based on this, we proposed that even small changes of *Foxg1* levels may result in a profound impact on pyramids' activity, an issue relevant to neuronal physiology and neurological aberrancies associated to *FOXP1* copy number variations. These findings have been published in Cerebral Cortex in 2020.

On the other side, we investigated the impact of *Foxg1* and *Emx2* on gliogenesis progression, and found that both inhibit such progression, by distinct mechanisms.

As anticipated by a study of ours published in 2010, *Foxg1* acts by antagonizing the transition from neocortical stem cells to early astrocyte/oligodendrocyte precursors. More recently, we readdressed this issue in living mice and reobserved the same phenotype. Moreover, we found that a similar control is exerted by *Foxg1* on primate gliogenesis. Finally, we identified key cellular and molecular mechanisms linking *Foxg1* to astrogenesis progression. These findings are the subject of a dedicated study published in Cerebral Cortex in 2019.

As for *Emx2*, we found that it rather acts *after* the conversion of neural stem cell to astrocyte progenitors, by promoting the exit of these progenitors from cell cycle and their differentiation to postmitotic astrocytes. In this way, it anticipates astroglial differentiation and limits the numerical astrogenic output originating from the starting stem pool. We reconstructed mechanisms, both cell-autonomous and not-cell-autonomous, linking *Emx2* overexpression to premature astroglial differentiation, and proved that timed decline of *Emx2* levels in late gestational neocortex is a prerequisite for the perinatal astrogenic burst. These results were published on *Glia* in 2015.

Gene therapy of glioblastoma multiforme.

Building on results of our study on *Emx2* control of astrogenesis, we subsequently showed that over-expression of this gene can be employed to suppress glioblastoma multiforme (GBM). Possibly as a consequence of its pleiotropic impact on GBM founder cell metabolism, *Emx2* turned out to be highly effective in a number of GBM of heterogenous origin, *in vitro* and *in vivo*. Remarkably, its overactivation doubled the survival time of nude mice orthotopically transplanted by human GBMs, outperforming TMZ. These findings were published on *Oncotarget* and were the basis of a patent application for gene therapy of this tumor. At the moment we are working at biosafe delivery of a therapeutic transgene *in vivo*.

RNA therapy of neuropathogenic haploinsufficiencies

Haploinsufficiency for specific genes and genesets underlie an impressive number of rare and severe neurological pathologies for which no cure is presently available. The huge heterogeneity of the corresponding pathogenetic mechanisms and their low individual prevalence make the development of effective cures for each of these pathologies a particularly challenging issue. How escaping this impasse? Unfortunately, neither homologous recombination-mediated repair of the defective chromosome/gene nor the employment of therapeutic minigenes are affordable options. We reasoned that the haploinsufficiency problem might be solved by stimulating the spared gene allele, driven by the whole array of regulatory elements which shape its normal spatio-temporal expression profile and finely tune its expression.

We successfully employed miRNA-like, small-activating RNAs (saRNAs) to stimulate transcription of *Emx2* and haploinsufficient *Foxg1*. We developed a novel class of RNA-programmable transactivators, the NMHVs, seven folds smaller than CRISPR-transactivators (CRISPR-TAs), suitable for transactivating endogenous genes ad libitum. Both saRNAs and NMHVs gave expression gains not far from 2 and quantifiable biological redouts. Differently from CRISPR-TAs, they complied with endogenous, natural regulation of their target genes. All that makes them a promising tool for therapy of haploinsufficiencies.

Beyond our early work on *Foxg1* and *Emx2* stimulation, we further developed saRNAs promoting transcription of *FXN* and *Scn1a* (haploinsufficiency for which underlie Friedreich ataxia and Dravet's syndrome, respectively).

E. Publication list

- Tigani W, Rossi MP, Artimagnella O, Santo M, Rauti R, Sorbo T, Ulloa FPS, Provenzano G, Allegra M, Caleo M, Ballerini L, Bozzi Y, Mallamaci A. Foxg1 Upregulation Enhances Neocortical Activity. *Cereb Cortex*. 2020 May 7;. doi: 10.1093/cercor/bhaa107. [Epub ahead of print] PubMed PMID: 32383447.
- Falcone C, Santo M, Liuzzi G, Cannizzaro N, Grudina C, Valencic E, Peruzzotti-Jametti L, Pluchino S, Mallamaci A. Foxg1 Antagonizes Neocortical Stem Cell Progression to Astrogenesis. *Cereb Cortex*. 2019 Dec 17;29(12):4903-4918. doi: 10.1093/cercor/bhz031. PubMed PMID: 30821834.
- Bon C, Luffarelli R, Russo R, Fortuni S, Pierattini B, Santulli C, Fimiani C, Persichetti F, Cotella D, Mallamaci A, Santoro C, Carninci P, Espinoza S, Testi R, Zucchelli S, Condò I, Gustincich S. SINEUP non-coding RNAs rescue defective frataxin expression and activity in a cellular model of Friedreich's Ataxia. *Nucleic Acids Res*. 2019 Nov 18;47(20):10728-10743. doi: 10.1093/nar/gkz798. PubMed PMID: 31584077; PubMed Central PMCID: PMC6847766.
- Chiola S, Santo M, Mallamaci A. Intraventricular Transplantation of Engineered Neuronal Precursors for In Vivo Neuroarchitecture Studies. *J Vis Exp*. 2019 May 11;(147). doi: 10.3791/59242. PubMed PMID: 31132045.
- Chiola S, Do MD, Centrone L, Mallamaci A. Foxg1 Overexpression in Neocortical Pyramids Stimulates Dendrite Elongation Via Hes1 and pCreb1 Upregulation. *Cereb Cortex*. 2019 Mar 1;29(3):1006-1019. doi: 10.1093/cercor/bhy007. PubMed PMID: 29385539.
- Desmaris E, Keruzore M, Saulnier A, Ratié L, Assimacopoulos S, De Clercq S, Nan X, Roychoudhury K, Qin S, Kricha S, Chevalier C, Lingner T, Henningfeld KA, Zarkower D, Mallamaci A, Theil T, Campbell K, Pieler T, Li M, Grove EA, Bellefroid EJ. DMRT5, DMRT3, and EMX2 Cooperatively Repress Gsx2 at the Pallium-Subpallium Boundary to Maintain Cortical Identity in Dorsal Telencephalic Progenitors. *J Neurosci*. 2018 Oct 17;38(42):9105-9121. doi: 10.1523/JNEUROSCI.0375-18.2018. Epub 2018 Aug 24. PubMed PMID: 30143575; PubMed Central PMCID: PMC6191521.

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- Mallamaci A. Enhancing Neuronogenesis and Counteracting Neuropathogenic Gene Haploinsufficiencies by RNA Gene Activation. *Adv Exp Med Biol*. 2017;983:23-39. doi: 10.1007/978-981-10-4310-9_2. PubMed PMID: 28639189.
- Fimiani C, Goina E, Su Q, Gao G, Mallamaci A. RNA activation of haploinsufficient Foxg1 gene in murine neocortex. *Sci Rep*. 2016 Dec 20;6:39311. doi: 10.1038/srep39311. PubMed PMID: 27995975; PubMed Central PMCID: PMC5172352.
- Falcone C, Daga A, Lanza G, Mallamaci A. Emx2 as a novel tool to suppress glioblastoma. *Oncotarget*. 2016 Jul 5;7(27):41005-41016. doi: 10.18632/oncotarget.9322. PubMed PMID: 27191499; PubMed Central PMCID: PMC5173038.
- Fimiani C, Goina E, Mallamaci A. Upregulating endogenous genes by an RNA-programmable artificial transactivator. *Nucleic Acids Res*. 2015 Sep 18;43(16):7850-64. doi: 10.1093/nar/gkv682. Epub 2015 Jul 7. PubMed PMID: 26152305; PubMed Central PMCID: PMC4652751.
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- Saulnier A, Keruzore M, De Clercq S, Bar I, Moers V, Magnani D, Walcher T, Filippis C, Kricha S, Parlier D, Viviani L, Matson CK, Nakagawa Y, Theil T, Götz M, Mallamaci A, Marine JC, Zarkower D, Bellefroid EJ. The doublesex homolog Dmrt5 is required for the development of the caudomedial cerebral cortex in mammals. *Cereb Cortex*. 2013 Nov;23(11):2552-67. doi: 10.1093/cercor/bhs234. Epub 2012 Aug 23. PubMed PMID: 22923088; PubMed Central PMCID: PMC3792737.
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F. PhD students graduated under AM supervision

PhD Course in "Molecular and Cellular Biology", HSR, Milan - Open University, London.

- (1) Luca Muzio (2005), thesis: "The role of Emx genes during the development of the mammalian cerebral cortex"

PhD Course in "Neuroscience-Neurobiology", SISSA, Trieste

- (1) Elisa Puzzolo (2009), thesis: "Cortico-cerebral development in the gray short-tailed opossum Monodelphis domestica"
 (2) Giulia Spigoni (2010), thesis: "Regulation of Emx2 expression by antisense transcripts in the murine developing CNS"
 (3) Marco Brancaccio (2010), thesis: "Emx2 and Foxg1 inhibit gliogenesis and promote neuronogenesis"

PhD Course in "Functional and structural Genomics", SISSA, Trieste

- (1) Nicola Antonio Maiorano (2009), thesis: "Promotion of embryonic cortico-cerebral neuronogenesis by miR-124"
 (2) Assunta Diodato (2010), thesis: "Promotion of cortico-cerebral precursors expansion by artificial miRNAs against the Emx2 locus"
 (3) Marilena Raciti (2012), thesis: "Reprogramming fibroblasts to neural-stem-like cells by structured overexpression of pallial patterning genes"
 (4) Carol Filippis (2013), thesis: "Emx2 expression levels in NSCs modulate astrogenesis rates by downregulating EgfR and Fgf9"
 (5) Mihn Duc Do (2014), thesis: "FoxG1 promotes neuritogenesis and the formation of dendritic spines – a potential mechanism for West syndrome"
 (6) Clara Grudina (2015), thesis: "The impact of Foxg1 on human cortico-cerebral astrogenesis"
 (7) Moira Pinzan (2016), thesis: "Modelling etiopathogenesis of the FOXG1-duplication-linked variant of West syndrome"
 (8) Carmen Falcone (2017), thesis: "Gene control of neocortical astrogliogenesis, in normal development and its disorders"
 (9) Cristina Fimiani (2017), thesis: "RNA therapy of neuropathogenic gene haplo-insufficiencies". *Qualified as the best 2017 PhD thesis of SISSA - Neuroscience Area.*
 (10) Simone Chiola (2018), thesis: "Foxg1 control of neuronal morphology"
 (11) Jessica Zucco (2019), thesis: "Towards Emx2 therapy of Glioblastoma multiforme"
 (12) Wendy Tigani (2019), thesis: "Functional and Molecular Impact of Foxg1 Over-expression in Neocortical Projection Neurons