MicroRNAs shape early neural fate within a critical time window in human brain organoids

Lisa Emmenegger1 , Cledi Alicia Cerda Jara1 , Matilde Ercolano2 , Jaden Loebert1 , Nicolas Morando3 , Poojashree Bhaskar1 , Denise Aigner1 , Ivano Legnini4 , Agnieszka Rybak-Wolf2 , Nikolaus Rajewsky5 1Laboratory for Systems Biology of Gene Regulatory Elements, Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine (MDC) in the Helmholtz Association, Berlin, Germany, 2Organoid Platform, Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany, 3 Instituto de Investigaciones Biomédicas en Retrovirus y Sida (INBIRS), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)- Universidad de Buenos Aires, Buenos Aires, Argentina, 4Human Technopole, Milano, Italy, 5Laboratory for Systems Biology of Gene Regulatory Elements, Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine (MDC) in the Helmholtz Association, Charité - Universitätsmedizin, German Center for Cardiovascular Research (DZHK), Berlin, Germany

Background: MicroRNAs (miRNAs) are highly abundant in the mammalian brain, yet their role in human neurodevelopment remains elusive due to the lack of physiologically relevant models. Method: Here, we used human induced pluripotent stem cell-derived forebrain organoids to characterize miRNA expression dynamics and function during early human neurogenesis. Results: We identified stage-specific miRNA expression signatures mirroring the temporal progression of corticogenesis, with late-stage profiles closely resembling those of the human fetal brain. We found that the abundance of both miRNAs and core components of the miRNA biogenesis machinery (DROSHA, DICER, AGO2) undergoes dynamic changes during development, peaking during neural commitment and declining with differentiation. To study miRNA function in neurogenesis, we globally perturbed miRNA biogenesis at distinct developmental stages using an inducible dominant-negative DROSHA mutant. Ablation of miRNAs prior to neural commitment impaired dorsal forebrain patterning, derepressed WNT and BMP signaling, and redirected cell identities toward cortical hem, midbrain and hindbrain fate. In contrast, post-commitment perturbations had only minor effects. Gain-of-function experiments identified specific miRNAs able to partially rescue the mispatterning. Conclusion: Collectively, these findings reveal a critical pre-commitment window in which miRNAs orchestrate forebrain specification and regional patterning, establishing miRNAs as essential regulators of early human brain development.