The role of astrocyte alterations in early changes in the dynamics of cultured cerebellar networks

Sivan Kanner[1], Miri Goldin[2a], Ronit Galron[1a], Yael Hanein[3ab], Eshel Ben Jacob[2a], *Paolo Bonifazi[2a], and ***Ari Barzilai**[1a]

[1]Department of Neurobiology, George S. Wise, Faculty of Life Sciences, [2]School of Physics and Astronomy, [3]School of Electrical Engineering, [a]Sagol School of Neuroscience, [b]Tel-Aviv University Center for Nanoscience and Nanotechnology, Tel-Aviv University, Ramat Aviv, Tel-Aviv 69978, Israel

An aberrant response to DNA lesions is implicated in many human brain degenerative disorders. Various types of DNA lesions activate a cellular process known as the DNA damage response (DDR). Mutations affecting the proteins involved in the DDR can lead to severe genomic instability syndromes that involve varying degrees of sensitivity to genotoxic stress, and also to tissue degeneration, cancer predisposition, and premature aging. Malfunctioning DDR was found in various brain degenerative disorders such as Alzheimer's, Parkinson's and Huntington. One of the key components of the DDR is the protein ATM, which is inactivated in the genomic instability disorder ataxia-telangiectasia (A-T). In order to study the effect of malfunctioning DDR on neuronal circuits, we used calcium imaging and immunocystochemical staining to compare the morphology and the dynamics of primary cerebellar cultures grown from postnatal Atm-deficient and wild-type (WT) mice. Cerebellar networks exhibited spontaneous network events after two weeks *in-vitro*. Compared to WT circuits, Atm-deficient circuits displayed a lower number of global synchronizations and a larger number of sparse synchronizations, i.e. synchronous events involving less than a dozen cells. In WT networks we observed significantly high global burst similarity compared to the Atm-/- network. In addition, nodes with a high functional connectivity degree could be observed in the WT networks but not in the Atm-/- networks. To understand A-T on the cellular level we tested the hypothesis that A-T is at least partially a glial disease. Immunocystochemical staining of astrocytes revealed a significantly less complex cell arborization in Atm-deficient versus WT circuits, as measured by the number of branches originating from cell bodies as well as their length. To further study the interrelations between neurons and astrocytes, we generated chimeric networks in which the neurons and astrocytes were extracted from different animals. We found that functional and viable chimera cultures could be prepared only from P8 cerebellar neurons and astrocytes. Chimera cultures made from combinations of P8 cerebellar neurons and P2 cortical glia or from P8 cerebellar neurons and P2 cerebellar glia did not survive and the neurons died within 3 to 4 days of plating. Our results clearly show that Atm-/- astroglial cell replacement with WT astrocytes fully restores the dynamics of neural networks in chimera neuron-glia networks extracted from Atm-deficient mice. In contrast, Atm-/- astrocytes failed to support the survival and the functionality of the WT neurons. These results support the notion that neuronal network failures in genetic brain degenerative diseases are correlated with impairment of astroglial cell functionality.