Study of the Mechanism of Tetraploid Astrocyte Generation in Pharmacoresistant Epilepsy using an In Vitro Model

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Abstract

Introduction: Epilepsy is a common chronic neurological disorder, and 25–33% of patients develop pharmacoresistant epilepsy (PRE). Its etiology remains unclear, highlighting the need to study molecular changes in the epileptogenic zone. Neurosurgical resections in PRE provide access to affected tissue, revealing an increased presence of tetraploid (4C) astrocytes. This study aims to explore their generation using an in vitro model to complement findings from neurosurgical samples

Method: The human astrocyte cell line CRL8621 was differentiated under serum-free conditions. Astrocyte markers (NDRG2, GFAP, GLUT1, AQP4, TrkB, GABA BR2, EAAT2, GABA T3, and P2X7) were assessed by immunocytochemistry in both differentiated and undifferentiated cells. Differentiated cells were exposed to three types of stimuli: trophic factors (NGF, BDNF, and their combination), pro-epileptogenic agents (LPS, kainate, bicuculline), and antiepileptic drugs (carbamazepine, lamotrigine, levetiracetam). Tetraploidy was quantified by flow cytometry following ethanol fixation and propidium iodide staining.

Results: Differentiated astrocytes retained their astrocytic markers. Significant differences were observed in the total percentage of cells with and without serum (N=8), with a lower number in the latter; however, there was a significant increase in diploid astrocytes and a decrease in those greater than 4C. The optimal time for treatment and fixation was found to be 48–72 h and 96 h, respectively. Regarding the trophic factor treatments (N=4), no significant differences were found, although a slight decreasing trend in diploid astrocytes was observed in the presence of BDNF. Similarly, treatments with pro-epileptogenic factors and antiepileptic drugs (N=4) did not produce significant changes in the proportion of tetraploid cells.

Discussion: A serum-free in vitro model using the CRL8621 astrocyte line was optimized to study tetraploid astrocytes in PRE. The results complement neurosurgical findings, showing that the tested stimuli did not alter the astrocyte cell cycle.

Conclusions: Further studies, ideally alongside neurosurgical tissue, are needed to identify factors influencing tetraploidy. Therefore, further complementary studies using this model — ideally in conjunction with neurosurgically obtained tissue — are needed to identify stimuli that influence tetraploidy.

References

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