GLIAL CELLS ANEUPLOID FROM CULTURE OF EQUINE NEONATE SPINAL CORD

Leandro Maia[1]
Ligia Souza Lima de Oliveira da Mota[2]
Fernanda da Cruz Landim-Alvarenga[3]
Renee Laufer-Amorim[4]
Bruna De Vita[3]
Carolina Nogueira de Moraes[3]
Rogério Martins Amorim[1]

ABSTRACT

The aim of this communication is to report the occurrence of glia cells aneuploid obtained from the culture of spinal cord of a newborn horse. Cells were maintained in culture until the sixth passage characterized by imunocytochemistry technique prior to cytogenetic analysis. Karyotype analysis showed loss or gain of one or more chromosomes in glial cells analyzed, when compared with the normal karyotype for equine specie. The occurrence of aneuploidy may be considered a normal finding in young neural cells, as well as a signal of several diseases. Thus, others cytogenetic studies should be performed to elucidate if the high occurrence of aneuploid glial cells in equine neonate nervous system is a physiological or pathological finding.

Keywords: nervous system, cell culture, cytogenetic, kariotype, aneuploidy, horse.

ANEUPLOIDIA EM CÉLULAS DA GLIA PROVENIENTES DA CULTURA DE MEDULA ESPINHAL DE EQUINO NEONATO

RESUMO

O objetivo desta comunicação é relatar a ocorrência de aneuploidia em células da glia provenientes do cultivo da medula espinhal de um equino neonato. As células foram mantidas em cultivo até a sexta passagem e caracterizadas pela técnica de imunocitoquimica previamente à análise citogenética. A análise do cariótipo revelou perda ou ganho de um ou mais cromossomos nas células gliais analisadas, quando comparado ao cariótipo normal para espécie equina. A ocorrência de aneuploidia pode ser considerada um achado normal em células jovens, bem como um sinal de enfermidade severa. Desta forma, outros estudos citogenéticos devem ser realizados para elucidar se a elevada ocorrência de aneulpoidia observadas em células da glia no sistema nervoso de neonato equino é achado fisiológico ou patológico.

Palavras-chave: sistema nervoso, cultura celular, citogenética, cariótipo, aneuploidia, cavalo.

[1] Department of Veterinary Clinics. School of Veterinary Medicine and Animal Science, São Paulo State University UNESP, Rubião Júnior, Botucatu, São Paulo, Brazil.
[2] Departament of Genetics. São Paulo State University UNESP, Rubião Júnior, Botucatu, São Paulo, Brazil.
[3] Department of Animal Reproduction. School of Veterinary Medicine and Animal Science, São Paulo State University UNESP, Rubião Júnior, Botucatu, São Paulo, Brazil.
[4] Department of Animal Pathology. School of Veterinary Medicine and Animal Science, São Paulo State University UNESP, Rubião Júnior, Botucatu, São Paulo, Brazil.

ANEUPLOIDÍA EN CÉLULAS DE LA GLIA PROVENIENTES DEL CULTIVO DE LA MÉDULA ESPINAL DE EQUINO NEONATO

RESUMEN

El objetivo de este trabajo es reportar la presencia de aneuploidía en células de la glía provenientes del cultivo de médula espinal de un neonato equino. Las células fueron mantenidas en cultivo hasta el sexto pasaje y caracterizadas por la técnica de inmunocitoquímica previamente a la realización de la citogenética. El análisis del cariotipo reveló pérdida o ganancia de uno o más cromosomas en las células gliales analizadas, en comparación con el cariotipo normal. La presencia de aneuploidía puede ser considerada un hallazgo normal en células jóvenes, bien como una señal de enfermedad severa. De esta forma, otros estudios citogenéticos deben ser realizados para esclarecer si la presencia elevada de aneuploidía en células de la glía del sistema nervioso de neonatos equinos constituye un hallazgo fisiológico o patológico.

Palabras clave: sistema nervioso, cultivo celular, citogenética, cariotipo, aneuploidía, caballo

Cytogenetics is a hybrid science that employs elements and methodology of genetics and cytology as well as from the analysis of the structure, morphology and behavior of chromosomes. The use of this technique allows to obtain information that helps to clarify issues related to reproduction, biology and evolution (1). The intercellular genomic variations that have probably the most appreciable impact on brain development and neurogenesis are related to aneuploidy or gain/loss of whole chromosomes. Affecting large proportions of neural cells, aneuploidy is usually devastative and it is suggested to be the hallmark of numerous pathogenic processes in human brain (2). However, according to Kingsbury *et al*. (3) the existence of aneuploid cells within the mammalian brain has suggested the influence of genetic mosaicism on normal neural circuitry. The objective of this scientific communication is to report the occurrence of glial cells aneuploid obtained from the spinal cord of a newborn horse and subjected to *in vitro* culture.

Samples from the spinal cord were obtained, during necropsy from the cervical region of a newborn female horse with 6 days of age. The samples were aseptically collected just after the animal death and placed in Falcon tubes (Techno Plastic Product® AG, Switzerland) with phosphate buffer solution (PBS - Nutricell®, Brazil) at a pH of 7.2.

At the laboratory the samples were fragmented with the help of a scalp blade (Two Arrrows, China) and subjected to enzymatic digestion with 0.4% Type 1 collagenase (Gibco, Grand Island, NY, USA) during one hour at 37ºC. Every 10 minutes the material was spin in a Vortex (Biomixer, USA). At the end of digestion period the cells were centrifuged for 10 minutes at 400 x g, and washed twice in Low Glucose DMEM (Dulbecco's modified medium, Gibco, Grand Island, NY, USA).

The cell suspension was divided into two 25 cm^2 culture bottles and the volume was completed to 5 mL of medium. The cells were cultured in Low Glucose DMEM/F12 (1:1) supplemented with 20% fetal calf serum, penicillin/streptomycin (1%) and anphotericin (1.2%). The material was incubated at 37.5ºC in a 5% CO_2 in air atmosphere with 95% humidity. Cellular growth was monitorised every 24 hours in a inverted microscope (Leica Microsystems, Germany) and the subcultures were performed when the cells achieved at least 80% confluence.

Cell culture was maintained until the 6^{th} passage and during this period samples were collected for imunocytochemistry characterization using markers for ***glial fibrillary acidic***

protein (GFAP) (Dako Cytomation, Denmark), S100Protein (Dako Cytomation, Glostrup, Denmark) and vimentin (Dako Cytomation, Glostrup, Denmark).

After the confirmation of the neural line, GFAP, vimentin and S100 protein positive, the glial cells from the 3nd and the 6th passage were subjected to the cytogenetic analysis using the technique of cell cycle synchronization with methotrexate (MTX) and thymidine. For the slides preparation cells were suspended with tripsin at 37.0ºC, fixed in 5% Giemsa and analyzed in a light microscope. For each passage 12 metaphases were analyzed.

The results showed aneuploid glial cells formed by the gain or loss of one or more chromosomes (Table 1) when compared with the species normal kariotype (2n= 64) (4).

Aneuploidy was also reported in studies performed in samples from the human (5-7) and mouse (3, 8) brain, supporting the data founded in the present study. The occurrence of aneuploidy may be considered normal in young neural cells, as well as a signal of several diseases. Consequently, others cytogenetic studies should be performed to elucidate if the high occurrence of aneuploid glial cells in equine neonate nervous system is a physiological or pathological finding.

The results of the present report may contribute to the study of genetic diseases and the clarification of the physiological mechanisms of the nervous system development, since it was previously shown the aneuploid neural cells can be functional (3).

Table 1. Diploid number of chromosomes founded in 12 metaphase obtained from a newborn horse glial cells cultured *in vitro* during the 3td, 4th, 5th, and 6th passage.

Metaphase	**Number of chromosomes 3rd passage (2n =)**	**Number of chromosomes 4th passage (2n =)**	**Number of chromosomes 5th passage (2n =)**	**Number of chromosomes 6th passage (2n =)**
1	57	64	60	56
2	59	58	64	64
3	64	63	59	62
4	56	62	64	62
5	60	64	64	61
6	59	60	62	64
7	62	64	62	59
8	64	64	64	56
9	62	59	65	62
10	60	64	66	56
11	61	64	64	58
12	55	63	64	64
Aneuploid proportion	**83,3%**	**50 %**	**50%**	**75%**

Normal kariotype for the equine species: 2n= 64 (Richer and others 1990).

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