Somatic tetraploidy in vertebrate neurons
Implications in physiology and pathology

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The presence of polyploid neurons in the vertebrate nervous system has been a subject of debate since the 1960s. At that time, Purkinje cells were proposed to be tetraploid, but technical limitations impeded to reach a clear conclusion, and the current belief is that most vertebrate neurons are diploid. By using up-to-date approaches we have recently demonstrated the existence of a subpopulation of tetraploid retinal ganglion cells (RGCs) in the vertebrate retina. In the chick, these neurons show large somas and extensive dendritic trees and most of them express a marker specific for RGCs innervating a specific lamina of the optic tectum. We have also demonstrated that these neurons are generated in response to nerve growth factor (NGF) acting through the neurotrophin receptor p75 (p75NTR), which induces E2F1 activity and cell cycle re-entry in migrating RGC neuroblasts lacking retinoblastoma (Rb) protein. We have also showed that brain-derived neurotrophic factor (BDNF) prevents G2/M transition in the tetraploid RGCs, thus being crucial for the maintenance of the tetraploid status as well as the survival of these neurons. The realization that tetraploid neurons can be readily observed in the vertebrate nervous system has important physiological consequences, which are discussed in this commentary.

Several eukaryotes are known to undergo endoreduplicative cycles leading to somatic polyploidy, thus increasing cell size in specific tissues. Examples of polyploidy in neurons are known in some invertebrates. In contrast, the vertebrate nervous system has classically been thought to be constituted by neurons with a diploid DNA amount. This belief was challenged when Lowell W. Lapham postulated in 1968 that Purkinje cells were tetraploid. Following this initial observation, several authors claimed that other large vertebrate neurons were also tetraploid. Nevertheless, many other studies questioned this concept and the absence of reliable procedures for DNA quantification made it impossible to reach a clear conclusion at that time.

Modern techniques such as flow cytometry, fluorescent in situ hybridization (FISH), slide-based cytometry (SBC) and quantitative PCR analysis of DNA from isolated nuclei can all reliably quantify the amount of nuclear DNA in neurons. By using SBC, flow cytometry and FISH, we have recently demonstrated that tetraploid neurons exist in the normal vertebrate retina, representing a subpopulation of RGCs that, in the chick, innervate lamina F in the stratum-griseum-et-fibrosum-superficiale of the tectal cortex. These neurons are generated during the early stages of retinal development, soon after they acquire neuronal markers. Indeed, a subset of migrating RGCs expressing the transcription factor E2F1, and lacking Rb protein, was observed to undergo S-phase and remain in a permanent G2-like state. Therefore, endoreduplication, but not alternative mechanisms such as aneuploidy or cell fusion, represents the mechanism generating tetraploid RGCs in the vertebrate nervous system.

Our work has demonstrated that endoreduplication in RGCs is triggered by NGF through p75NTR, since blocking...
antibodies against these molecules were able to prevent cell cycle re-entry and tetraploidy in the retinal neurons.12 Furthermore, activation of p75NTR in differentiating retinal neurons was observed to induce E2F1 activity.13 These results are consistent with the observed decrease of BrdU incorporation described in the developing retina of p75NTR knock-out mice.15 As far as we know this is the first time in which a signaling mechanism inducing endoreduplication in vertebrates has been revealed.

Both NGF and p75NTR are known to fulfill several functions in the nervous system including cell cycle regulation,16 and several intracellular interactors of p75NTR have been shown to modulate cell cycle progression.16 Among them, different members of the Melanoma Antigen (MAGE) protein family have been shown to mimic the E2F1-blocking effect of Rb,17 thus being putative regulators of the postmitotic state of RGCs lacking Rb expression. CMAGE, the chicken MAGE protein, is known to co-localize with p75NTR in the developing RGCs,17 thus representing a candidate protein for the regulation of endoreduplication in these neurons. p75NTR can release its intracellular domain (p75ICD) in response to ligand binding by means of a γ-secretase-dependent mechanism,16 and the p75ICD peptide has been shown to facilitate E2F1 activity by preventing the blocking effect of CMAGE.17 This suggests that endoreduplication may result from the activation of a p75ICD-containing peptide that subsequently blocks CMAGE, thus facilitating E2F1 function in postmitotic RGCs lacking Rb expression (Fig. 1). This model is further supported by a recent report demonstrating that p75ICD can interact with the promoter of cyclin E1,19 a known cell cycle regulator crucial for endoreduplication,1,2 which is expressed in response to E2F1 activation.

Endoreduplication is characterized by DNA synthesis in the absence of cell division. In this regard, we have shown that most migrating RGCs that reactivate the cell cycle remain in a G1-like state.12 The neurotrophin BDNF is likely involved in preventing G1/M transition in tetraploid RGCs since it has been shown to inhibit cyclin B1 expression and ectopic mitotic figures in NGF-treated, differentiating retinal cells.20 Blockade of BDNF in embryonic eye explants resulted in an increase of differentiating RGCs undergoing mitosis,12 thus indicating that endogenous levels of BDNF prevent G1/M transition in tetraploid RGCs. The mechanism used by BDNF to achieve this effect is currently unknown.

Differentiating tetraploid RGCs that undergo mitosis finally die.12 This conclusion was evidenced by means of BrdU pulse and chase experiments in vivo, which allowed us to analyze the fate of migrating neuroblasts that undergo cell cycle re-entry. While most of these cells remained in the retina with a 4C DNA content, a minority of them were observed to undergo mitosis followed by apoptosis, as previously observed in vitro.20 This finding indicates that the induction of apoptosis by p75NTR in the RGC neuroblasts depends on the completion of mitosis in these cells, as previously suggested.20 At present, the molecular mechanism triggering apoptosis in RGCs that undergo mitosis remains to be determined.

Tetraploid RGCs in the chick show increased soma size and extensive dendritic trees,12 thus sharing features with primate parasol cells and α-Y cells in the cat.22 These neurons are involved in motion processing and show a specific distribution pattern along the ganglion cell layer (GCL). Therefore, adjusting the numbers of large vs. small RGCs may be crucial to assure the proper functioning of the retina. In this regard, the density of large RGCs is known to be increased in the peripheral retina,23 whereas apoptosis becomes increased in the center of this tissue.24 This suggests that early cell death in the central retina is required to reduce the number of tetraploid RGCs and increase the ratio between small and large RGCs in this region. TGFβ might participate in the removal of tetraploid RGCs as it has been shown to cooperate with NGF in triggering retinal apoptosis,26 mainly acting on large RGCs.27

Cell cycle re-entry in neurons has classically been a synonym of apoptosis.28 Our data indicate that reactivation of the cell cycle can also be interpreted in terms of neuronal tetraploidy. This extends the significance of cell cycle re-entry in neurodegeneration since it may also result in tetraploidization,21 followed by neuronal hypertrophy,29 which may result in altered neuronal function and changes in neuronal circuits leading to neuronal degeneration.30
References

1. Edgar BA, Orr-Weaver TL. Endoreplication cell cycles: more for less. Cell 2001; 105:297-306.

2. Ullah Z, Lee CY, Lilly MA, DePamphilis ML. Developmentally programmed endoreduplication in animals. Cell Cycle 2009; 8:1501-9.

3. Coggeshall RE, Yaksta BA, Swartz FJ. A cytophotometric analysis of the DNA in the nucleus of the giant cell, R-2, in Aplysia. Chromosoma 1970; 32:285-12.

4. Manfredi Romanini MG, Fraschini A, Bernocchi G. DNA content and nuclear area in the neurons of the cerebral ganglion in Helix pomatia. Ann Histochim 1973; 18:49-58.

5. Swift H. Quantitative aspects of nuclear nucleoproteins. Int Rev Cytol 1953; 2:1-76.

6. Lapham LW. Tetraploid DNA content of Purkinje neurons of human cerebellar cortex. Science 1968; 159:310-2.

7. Herman CJ, Lapham LW. DNA content of neurons in the cat hippocampus. Science 1968; 160:537.

8. Herman CJ, Lapham LW. Neuronal polyplody and nuclear volumes in the cat central nervous system. Brain Res 1969; 15:35-48.

9. Museridze DP, Svanidze IK, Macharashvili DN. Content of DNA and dry weight of the nuclei of neurons of the external geniculate body and retina of the eye in guinea pigs. Proc Natl Acad Sci USA 2010; 107:109-14.

10. Frade JM, Escol P, de la Hera A, Frade JM. Somatic tetraploidy in specific chick retinal ganglion cells induced by nerve growth factor. Proc Natl Acad Sci USA 2010; 107:109-14.

11. Kingsbury MA, Yung YC, Peterson SE, Westra JW, Chiu J. Aneuploidy in the normal and diseased brain. Cell Mol Life Sci 2006; 63:2626-41.

12. Ying QL, Nichols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. Nature 2002; 416:545-8.

13. Harada C, Harada T, Nakamura K, Sakai Y, Tanaka K, Parada LF. Effect of p75<sup>−/−</sup> on the regulation of naturally occurring cell death and retinal ganglion cell number in the mouse eye. Dev Biol 2006; 296:57-65.

14. López-Sánchez N, Frade JM. Control of the cell cycle by neurotrophins: lessons from the p75 neurotrophin receptor. Histol Histopathol 2002; 17:1227-37.

15. López-Sánchez N, González-Fernández Z, Niinobe M, Yoshikawa K, Frade JM. Single mage gene in the chicken genome encodes CMage, a protein with functional similarities to mammalian type II Mage proteins. Physiol Genom 2007; 30:156-71.

16. Frade JM. Nuclear translocation of the p75 neurotrophin receptor cytoplasmic domain in response to neurotrophin binding. J Neurosci 2005; 25:1407-11.

17. Parkhurst CN, Zampieri N, Chao MV. Nuclear localization of the p75 neurotrophin receptor cytoplasmic domain in response to neurotrophin binding. J Neurosci 2005; 25:1407-11.

18. Parkhurst CN, Zampieri N, Chao MV. Nuclear localization of the p75 neurotrophin receptor cytoplasmic domain in response to neurotrophin binding. J Neurosci 2005; 25:1407-11.

19. Faraday JM, Rodriguez-Tébar A, Barde YA. Induction of cell death by ecdysone in nerve growth factor through its p75 receptor. Nature 1996; 383:166-8.