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Review

The genomically mosaic brain: Aneuploidy and more in neural diversity and disease

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ABSTRACT

Genomically identical cells have long been assumed to comprise the human brain, with post-genomic mechanisms giving rise to its enormous diversity, complexity, and disease susceptibility. However, the identification of neural cells containing somatically generated mosaic aneuploidy – loss and/or gain of chromosomes from a euploid complement – and other genomic variations including LINE1 retrotransposons and regional patterns of DNA content variation (DCV), demonstrate that the brain is genomically heterogeneous. The precise phenotypes and functions produced by genomic mosaicism are not well understood, although the effects of constitutive aberrations, as observed in Down syndrome, implicate roles for defined mosaic genomes relevant to cellular survival, differentiation potential, stem cell biology, and brain organization. Here we discuss genomic mosaicism as a feature of the normal brain as well as a possible factor in the weak or complex genetic linkages observed for many of the most common forms of neurological and psychiatric diseases.

Contents

1. Introduction

Aneuploidy is a gain (hyperploidy) or loss (hypoploidy) of chromosomes such that the resulting chromosome number is not an exact multiple of the haploid complement. A related term, aneusomy, reflects specific chromosome gains (hypersomy) or loss (hyposomy) in a cell, although the full karyotype for that cell may be unknown relative to the germline chromosomal complement. Aneuploidies and aneusomies within an organism can be defined as either *constitutive*, meaning that changes begin in the germline or early embryogenesis, resulting in a conserved change in virtually all cells of an organism; or *mosaic*, which indicates somatic changes in individual cells that result in mixed aneuploid and euploid forms with varied prevalence throughout an organism. There are several well-known pathophysiological chromosomal disorders including Down (trisomy 21), Edwards (trisomy 18), and Patau (trisomy 13) syndromes, which are most commonly constitutive in >95% of cases

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[1–5], along with sex chromosome aneuploidies like Klinefelter's (XXY) and Turner's (monosomy of X) syndromes that also result in abnormal development and behavior [6–10]. Mosaic disorders affecting the brain have also been described, such as mosaic variegated aneuploidy (MVA) [11–15].

While such chromosomal aberrations have been long associated with neurogenetic disorders, chromosomal aneuploidies or aneusomies are also known to be a normal feature of the brain, manifesting as complex mosaics [16–28]. In the central nervous system (CNS), mosaic aneuploidies were first identified in the cerebral cortex of normal developing mice [23], a result that has been extended throughout the neuraxis and to all vertebrate species thus far examined [21,22,25], including non-diseased humans [19,24,25,27,28]. Moreover, these changes have been a harbinger for other genomic alterations, generally referred to as DNA content variation (DCV) [29] 2010). Here we discuss genomic mosaicism in the non-diseased brain, and how it may contribute to human brain diseases.

2. Genomic diversity in cells of the normal brain: mosaic aneuploidy and DNA content variation (DCV)

2.1. Detection techniques

As early as 1902, Theodor Boveri identified chromosome aberrations in cancerous tumors, demonstrating the existence of living, aneuploid cells [30]. The simplest evaluations of chromosome numbers merely count chromosomes in metaphase spreads, when the condensed state of the chromatids allows for visualization, as well as identification of balanced and unbalanced translocations by Giemsa staining [31]. Despite the simplicity of this assay, it is notable that the correct human complement of chromosomes was not established until 1956 [32], some three years after report of the double helix [33], underscoring ambiguities that are associated with chromosome counts. A definitive modern technique called spectral karyotyping, or SKY, relies on the hybridization of genomic fragments labeled with distinct fluorochromes to the metaphase spreads of single cells and the subsequent identification of each chromosome pair or sex chromosomes [34] (Fig. 1A). These strategies require condensed chromosomes, and as such cannot be definitively used on interphase or non-mitotic cells. Fluorescent *in situ* hybridization (FISH) also employs hybridization of a probe against a defined but limited chromosomal region ("point probes"), which can be used to assess aneusomies in single interphase cells using a fluorescent or enzymatic readout (Fig. 1B). Multicolor FISH allows for simultaneous evaluation of several chromosomes or different regions along a single chromosome, including quantification of FISH signal intensity [35]. However, there are technical limitations that can lead to false-positive and false-negative probe hybridization, which require careful controls to identify true aneuploidy *versus* artifactual hybridization, such as pairing of chromosome homologs that may lead to the incorrect interpretation of a "pseudo monosomy" [27]. A modification of point probe FISH is interphase chromosome-specific multicolor banding (ICS-MCB) wherein a set of specific paints derived from microdissected chromosomes labels the target chromosome with a distinct spectral pattern for the simultaneous visualization of several regions of the chromosome [36,37]. This technique has not been widely used and may depend on the cell type and/or age of the interrogated chromatin. An independent technique for chromosomal copy number analysis is comparative genomic hybridization (CGH) and array CGH [38,39]. CGH requires the hybridization of test genomic samples to a representation of a standardized genome, and allows for copy number analyses from tissue samples or prenatal cytogenetic samples. Previously, the requirement of a relatively large, genomically homogenous set of cells limited the use of CGH in identifying mosaic aneuploidy. While Ballif and colleagues reported the detection of mosaicism even at levels of 10–20% [40], its effectiveness in CNS samples remains to be determined.

Single cell approaches that are currently in development will help to lower the detection threshold. The genome from single cells isolated by laser microdissection, flow cytometry, or other techniques could be amplified in a uniform and unbiased manner (*e.g.*, using multiple displacement amplification (MDA) [41]) for analysis by single-cell CGH or quantitative PCR for target genomic regions. Even more definitively, the resulting amplicons from single-cell MDA could serve as a template for genomic sequencing, an approach being pursued for cancer cells [42,43], as well as partial sequencing from neurons [44]. The promise of these techniques is currently tempered by a range of factors including use of adequate control genomes, the current low throughput of the technique that is critical in view of the one trillion cells that make up the human brain, and sufficient information storage limitations for the terabytes of data produced by whole-genome sequencing.

A distinct approach to assessing genomic uniformity is DNA flow cytometry that has a long history of identifying cells with varying DNA content associated with phases of the cell cycle [45,46]. The highly integrated and physically connected nature of the brain (*e.g.*, its synaptic neuropil) makes analyses of single cells difficult and incomplete, thus limiting prior flow cytometry efforts for studying the brain. Modifications of this approach to interrogate isolated nuclei rather than intact cells from the brain for DNA content (Fig. 1C) has identified brain cell populations with a surprising range of DNA content (Fig. 1D). This was manifested as an overall increase in DNA content within cerebral cortical neurons compared to cerebellar neurons from the same individual, demonstrating the pervasive existence of normal human brain cells having DNA content variation (DCV) (Fig. 1E) [29]. DCV in the frontal cortex averages a gain of 250 Mb, with NeuN-positive neurons showing significant increases compared to non-neuronal nuclei. Importantly, DCV appears to encompass myriad forms of mosaic aneuploidy that exist in both the cerebral cortex and cerebellum [24,25,27,28]. By contrast, DCV also appears to be distinct from aneuploidy because of the expanded DNA content histograms in the cerebral cortex that are less prominent in the cerebellum, suggesting an independent mechanism for increased DNA content.

These technical approaches, along with others in development, have allowed assessments of single brain cells, demonstrating genomic mosaicism amongst cells of the brain – and likely other tissues and cells, including stem cell lines [47,48] – thus redefining the genomic organization of the brain from homogenously uniform to a complex genomic mosaic. These data underscore a need to consider individual genomes in cellular function in the normal and diseased brain, as well as the effects of identified genes operating in varied genomic surroundings.

2.2. Mosaic aneuploidy in the non-diseased brain

The first report of widespread genomic mosaicism came from studies of aneuploidy in mice, which revealed that approximately 33% of proliferating cerebral cortical neural progenitor cells (NPCs), isolated from the ventricular zone of the embryonic brain [23], were aneuploid. A range of other neurogenic regions generate aneuploid cells, including cerebellar NPCs that represent ~15% of mitotic cells at postnatal day (P) P0 and ~21% at P7 [17,25]. This somatically derived form of genomic variation is characterized by the apparently stochastic loss or gain of all chromosomes, creating a genomic mosaic that displays a predominance of hypoploidy over hyperploidy [23]. During periods of cell division, mosaic aneuploidy in NPCs results from chromosomal segregation defects (lagging chromosomes, non-disjunction and supernumerary centrosomes)