Mini-Symposium

Down Syndrome: From Understanding the Neurobiology to Therapy

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Down syndrome (DS) is the most common example of a neurogenetic aneuploid disorder leading to mental retardation. In most cases, DS results from an extra copy of human chromosome 21 producing deregulated gene expression in brain that gives raise to subnormal intellectual functioning. Understanding the consequences of dosage imbalance attributable to trisomy 21 (T21) has accelerated because of recent advances in genome sequencing, comparative genome analysis, functional genome exploration, and the use of model organisms. This has led to new evidence-based therapeutic approaches to prevention or amelioration of T21 effects on brain structure and function (cognition) and has important implications for other areas of research on the neurogenomics of cognition and behavior.

Introduction

Trisomy for human chromosome 21 (Hsa21) is the most frequent live-born aneuploidy and results in Down syndrome (DS). This is a well recognized syndrome with variable phenotypic expression. DS results in cognitive impairment, dysmorphic features, and a number of mostly nonspecific manifestations for which severity and frequency are highly variable among different individuals.

In DS, deficits in learning, memory, and language lead to a general cognitive impairment, which is typically in the mild-to-moderate range. Morpho-syntax, verbal short-term memory, and explicit long-term memory are usually more impaired, whereas visual–spatial short-term memory, associative learning, and implicit long-term memory are better preserved (for review, see Lott and Dierssen, 2010). However, there is broad phenotypic variability among individuals with DS, and this is likely the result in part of genetic and epigenetic variation, environmental factors, and stochastic events. Individuals with DS have reduced brain volume with disproportionally smaller volumes in frontal and temporal areas and cerebellum. Lenticular nuclei as well as posterior parietal and occipital cortical gray matter are relatively preserved. For unknown reasons, the parahippocampal gyrus appears larger in DS than in the typical population. Recent evidence also points to a nonmotor contribution from the cerebellum. Seizures occur in a bimodal distribution with ~50% occurring before 1 year of age and 50% occurring in mid-adult life, and are considered to reflect structural brain abnormalities including a paucity of inhibitory neurons and defects in cortical lamination. The latter often herald the development of dementia, and our data suggest that the presence of seizures during the early phase of dementia is associated with poor cognitive performance and rapid decline (I. Lott et al., unpublished observations). These features may be dependent on morphogenetic alterations, but also on a reduced remodeling potential and impaired neural plasticity (Dierssen et al., 2009).

DS presents a unique platform for studying changes of brain aging across the lifespan. By age 40 years, there is a ubiquitous occurrence of plaques and tangles suggestive of Alzheimer disease (AD), but dementia is variable. The earliest manifestations of dementia in DS appear to reflect frontal lobe dysfunction with changes in sociability, emotional-based language, and depressive symptoms. However, physical–chemical dating of amyloid has suggested that it is first deposited in the frontal and entorhinal cortices. The amyloid burden in DS is, in part, related to increases in the expression of the amyloid precursor protein gene, but other factors are likely involved. Oxidative stress secondary to

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Links: Kyoto Encyclopedia of Genes and Genomes, KEGG: http://www.genome.jp/kegg/; Reactome: http://www.reactome.org/; Pathway Interaction Database, PID: http://pid.nci.nih.gov/; Human Protein Reference Database, HPRD: http://www.hprd.org/; Biological General Repository for Interaction Datasets, BioGRID: http://thebiogrid.org/; The IntAct molecular interactions database: http://www.ebi.ac.uk/intact/main.xhtml; Online Mendelian Inheritance in Man, OMIM: http://www.ncbi.nlm.nih.gov/omim.

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critical-region mutations in mitochondrial DNA is associated with an increased brain concentration of oxidized β-amyloid. A current focus of research is the identification of neurophysiological, imaging, and biomarker abnormalities in plasma and CSF that might predict cognitive decline in DS. Such observations are important in the timing of interventions to possibly improve cognitive functioning and to prevent dementia. Before evidence of dementia, neurite sprouting occurs in hippocampal areas in adults with DS, suggesting a possible compensatory response. Increased glucose metabolic rate has been seen in medial temporal lobe structures in nondemented adults with DS, the same areas that are hypometabolic in patients with AD in the general population.

Gene expression and trisomy 21

Despite the prevalence of DS, relatively few resources have been mobilized to support research into understanding its neurobiology or developing therapeutics for cognitive deficits. This neglect has been attributed in part to the presumed global nature of the molecular and cellular abnormalities resulting from trisomy 21 (T21), which involves misexpression of hundreds of genes in every cell throughout life. Several "dosage-sensitive" regions, including genes and noncoding conserved elements, have been mapped across the length of Hsa21 and shown to be sufficient for induction of the complete phenotype of DS (Korbel et al., 2009; Lyle et al., 2009). Exciting new findings are demonstrating the considerable plasticity of the human genome, and, in addition to direct and indirect alteration of expression of Hsa21 and non-Hsa21 genes, we have to consider that the variability of the DS phenotype may also be a result of copy number alteration of functional, nontraditional genomic elements. The question of the altered transcriptome in T21 has been investigated by microarray and real-time PCR experiments (Prandini et al., 2007). These studies confirmed that the genes on chromosome 21 are overexpressed for the most part in the expected dosage-specific ratio corresponding to gene copy number (i.e., ~50% higher than euploid). The preponderance of studies now suggests that expression of non-chromosome 21 genes is affected as well, although the degree to which this effect results from shifts in cell populations in a given tissue remains to be determined. Although this is likely the result, in part, of natural expression variation among diploid individuals (Antonarakis et al., 2004), it severely complicates interpretation of expression differences in DS. High-throughput RNA sequencing (digital measurement of RNA molecules per transcript) has been used to study monozygotic twins discordant for T21, providing evidence for a relevant number of metabolic and developmental pathways that are specifically disturbed in DS. The results of such studies (Antonarakis et al., unpublished observations) provide initial evidence supporting the hypothesis that some of the phenotypes of DS are attributable not to specific genes, but rather to the overexpression or underexpression of a whole chromosomal domain. DS should thus be viewed as a prototype "genomic disorder" and an excellent model for application of system neuroscience approaches.

Pathways to intellectual disability in Down syndrome

Hsa21 encodes ~160 "classical" protein-coding genes annotated in the SwissProt database, 5 microRNAs, and an additional >350 genes of unassigned function (Gardiner et al. unpublished observations). Overexpression of these genes, as in Down syndrome, will result in complex perturbations of multiple processes involved in neurological development and function. A systems neuroscience, pathway-based approach is being used, not only to understand non-Hsa21 molecular abnormalities observed in DS and mouse models, but also to predict additional abnormalities and potential responses of these systems to drug treatments. The subset of pathways relevant to intellectual disability (ID) in DS are termed DS-ID pathways and are defined as those pathways with components and/or interacting proteins that include Hsa21 proteins and one or more ID proteins, proteins known to be involved in ID from mutation analysis in human subjects. Inspection of the resulting set of DS-ID pathways provided significant observations. (1) Few pathways include Hsa21 proteins as components; rather DS-ID pathways are heavily impacted by interactions with Hsa21 proteins. (2) Perturbations in nerve growth factor and Sonic Hedgehog (SHH) signaling, observed in the Ts65Dn, the mouse model most often used in DS studies (Cooper et al., 2001; Roper et al., 2006), are predicted from pathway associations of ID and Hsa21 components and interactions. (3) Perturbations of similar significance are predicted in glucocorticoid, NOTCH, and Wnt signaling, and caspase cascades in apoptosis, among others. (4) A small number of Hsa21 proteins, including APP, TIAM1, ITSN1, SUMO3, ITGB2, and S100B, impact a large number of pathways. (5) No pathway is impacted by a single Hsa21 protein, emphasizing the need for DS model systems over expressing complex sets of genes. (6) The majority of pathways, including those listed above, are influenced by proteins mapping throughout Hsa21 and include those whose orthologs map proximal to the Ts65Dn breakpoint on mouse chromosome (Mmu) 16 and to Mmu10, emphasizing the likelihood that the molecular basis of drug responses in the Ts65Dn will be different in human DS (pathway data are available and searchable at http://gfuncpathdb.ucdenver.edu/iddrc/hsa21gdpw/home.htm). Experimental comparison of pathway perturbations in the Ts65Dn and Tc1 mouse models provides further support for the efficacy of the pathway approach.

Modeling Down syndrome in mice

The genetic dependence of the cognitive phenotype in DS is recapitulated in mouse models of the disorder (Dierssen et al., 2009). In the early 1990s, the generation of a genetic mouse model for DS by Muriel Davisson provided the basis for demonstrating that trisomy for the same genes has some closely related structural and functional outcomes in mouse and human (Reeves et al., 1995). Key among these are changes in the brain, which provide the substrate for focused efforts toward improving cognition based on specific alterations to hippocampal function in T21. There are four particular areas of interest. First, there is an imbalance of modulatory inputs to hippocampus such that inhibitory interneurons overbalance excitatory inputs (Kurt et al., 2000, 2004; Hanson et al., 2007), providing druggable targets to correct one key physiological imbalance in trisomic brains (Fernandez et al., 2007). Second, all individuals with T21 develop plaques and tangles of AD by their fourth decade. Third, individuals with DS have a significantly small cerebellum. The origins of this reduced growth have been traced to the time, cell type, cell process, and growth factor involved, showing that trisomic granule cell precursors have an attenuated mitogenic response to the SHH growth factor. Treatment of trisomic mice with an agonist of the SHH pathway restores cerebellum to normal with surprising salutary effects on brain function. Fourth, the Ts65Dn show altered structural plasticity, suggesting that also after the gene-driven developmental period experience-dependent neuronal shaping is altered, thus compromising the flexibility of the neural control of behavior in the face of a varying environment (Martínez-Cué et al., 2002, Dierssen et al., 2003). In the last years,

several groups created a series of new mouse models to dissect the Hsa21 gene interactions generating the DS phenotype. Orthologous genes on Hsa21 are located on Mmu16, 17, and 10. The DS critical region was clearly shown to be required for the DS-induced phenotype observed in the Ts65Dn mice but to be insufficient by itself to cause the complete set of DS traits (Olson et al., 2004; Belichenko et al., 2009). The Hsa21 transchromosomic (Tc1) mice, carrying an almost complete Hsa21, have a phenotype that strongly impacts locomotor activities and less severely affects cognitive phenotypes (O'Doherty et al., 2005; Morice et al., 2008; Galante et al., 2009), compared with the well studied Ts65Dn. Additional models have been generated with partial trisomies and monosomies for different regions orthologous to Hsa21 (Besson et al., 2007; Li et al., 2007; Pereira et al., 2009; Yu et al., 2010a,b). The first animals carrying trisomic segments covering the three regions orthologous to Hsa21 were described with DS phenotypes similar to Tc1 but surprisingly weaker than Ts65Dn (Yu et al., 2010b). Overall, these studies illustrate the extreme complexity of the genetic basis of DS phenotypes. Although they support the idea that DS phenotypes are the result of the contribution of multiple loci located throughout Hsa21, they also clearly show that interactions among trisomic genes are complex with additive, subtractive, or even epistatic contributions (Pereira et al., 2009). Now that a comprehensive set of trisomic and monosomic mouse models for DS is available, regional contributions to the induction of DS phenotypes can be determined to find dosage-sensitive genes, to better understand the pathophysiology of the trisomy, and to propose new therapeutic approaches to ameliorate the consequences of DS.

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