Neuroscience and Biobehavioral Reviews 71 (2016) 323–327

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

Review article

Epigenomic engineering for Down syndrome

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ARTICLE INFO

Article history:
Received 16 March 2016
Received in revised form 11 August 2016
Accepted 15 September 2016
Available online 16 September 2016

Keywords:
Down syndrome
Aneuploidy
Epigenomics
Genome editing
CRISPR-Cas9

ABSTRACT

Down syndrome (DS; trisomy 21), the commonest genetic cause of mental disability, affects approximately 250,000 families in the United States alone. Despite milestones in understanding the specific genetic causes of the syndrome, the major symptoms of DS – not least those related to neurocognitive function – are incurable. DS phenotypes are highly variable, and gene expression patterns cannot be explained by trisomy alone, implicating epigenetics in DS pathophysiology. DNA and histone modifications appear to contribute to DS pathology and cognitive defects, and epigenomic, and genome editing research have very recently opened up novel therapeutic avenues for several diseases including DS. Here, we discuss how epigenomic therapies might be used to ameliorate DS-related phenotypes with a particular focus on the CRISPR-Cas 9 system for targeted epigenomic engineering in DS. This approach is likely to reap rewards in terms of understanding the pathophysiology of DS, especially when combined with animal models, but significant technical and ethical challenges must be overcome for clinical translation.

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1. Introduction

Down syndrome (DS) (or trisomy 21) affects approximately 250,000 individuals in the United States (US) alone and represents the commonest genetic cause of mental disability (Sullivan et al., 2007). Despite longstanding interest in developing gene therapies for disorders such as cystic fibrosis or muscular dystrophy, other non-Mendelian genetic disorders such as DS have not received the same attention, partly due to their biological and phenotypic complexity. There have, however, been advances in the treatment of DS-related morbidity – for example, cardiac surgery for congenital heart disease – resulting in impressive increases in life expectancy from 25 years in 1983 to 49 years in 1997 in the US, with overall life expectancy in the developed world now averaging over 55 years (Weijerman and De Winter, 2010; Wiseman et al., 2009). Nevertheless, there remains room for improvement in both quality of life and mortality outcomes in these individuals.

Current DS treatment is directed toward mitigating neonatal complications (congenital cardiac and gastrointestinal problems and respiratory infections) and preventative health measures (Weijerman and De Winter, 2010). Mental retardation and dementia, the commonest neurological complications of DS, remain a therapeutic challenge. Children with DS have impaired language skills, learning difficulties, and both short- and long-term memory deficits (Roper and Reeves, 2006), although phenotypes are highly variable. In later life, many patients suffer from early-onset dementia from Alzheimer's disease (Zigman and Lott, 2007). Although this latter phenotype is thought to be caused by a gene dosage effect caused by the extra copy of the amyloid precursor protein (APP) gene on the third chromosome 21 to produce the characteristic amyloid plaques seen in Alzheimer's disease, only 50–70% of individuals with DS develop the disease (Zigman and Lott, 2007). The heterogeneous phenotypes seen in DS suggest that, in spite of

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http://dx.doi.org/10.1016/j.neubiorev.2016.09.012
0149-7634/

triplication of chromosome 21 (HSA21) in all patients, gene action in DS may not simply be a product of gene dosage independent of the environment, other genes, or transcriptional regulation, but instead result from epigenomic phenomena, i.e., falls under the control of the molecular regulators of gene function such as DNA and histone proteins. In support of this, only 22% of genes expressed in DS lymphoblastoid cells show 1.5-times increase in expression expected by trisomy alone, with the majority (56%) showing repression (Ait Yahya-Graison et al., 2007), and microarray-based gene expression profiling of adult DS brains implicates chromatin remodeling genes in DS (Lockstone et al., 2007). Further elegant studies in monozygotic twins discordant for DS have shown genome-wide alterations in gene expression extending beyond chromosome 21 and suggesting chromatin-related epigenetic modifications (Letourneau et al., 2014). It, therefore, follows that the epigenetic modifications that affect the global transcriptome in DS may be suitable targets for therapy.

Here we provide a short overview of the role of epigenetics in the pathophysiology of DS and how it might be exploited for therapeutic benefit. In particular, we focus on how new gene editing systems can be used therapeutically against epigenetic targets to overcome the neurological deficits seen in individuals with DS. We also outline some of the anticipated challenges in using this technology in the future for clinical purposes.

2. Epigenetics and the neurobiology of Down syndrome

The exponential growth of epigenome research and data from high-throughput approaches has opened up novel therapeutic avenues for several diseases (Roadmap Epigenomics et al., 2015), including neurological and genetic disorders. It is evident that learning and memory, which are both impaired in many individuals with DS, can be modulated by epigenetic mechanisms (for an excellent review on the topic, see Dekker et al. (2014)).

Epigenetics is defined as a heritable phenotype resulting from chromosomal changes without alterations in the DNA sequence (Berger et al., 2009). Chromatin is formed from DNA, histones, and non-histone proteins, with each nucleosomal unit formed from 146 DNA base pairs wrapped around a histone octamer (two of H3, H4, H2A, and H2B) (Luger et al., 1997). Since the local structure of chromatin determines accessibility to the gene expression control machinery (which is generally accessible in open euchromatin and inaccessible in compact heterochromatin), mechanisms that remodel the chromatin orchestrate transcription. There are five main remodeling processes: DNA methylation, post-translational histone modifications, nucleosomal positioning, histone variant incorporation, and the action of small noncoding RNAs (microRNAs (miRNAs) and long noncoding RNAs (lncRNAs)) (Weber et al., 2015). Of these, recent "cognitive epigenetic" research (Day and Sweatt, 2011) has shown that DNA methylation, post-translational histone modifications, and small noncoding RNAs, in particular, participate in neurodevelopment, synaptic plasticity, and learning and memory (Dekker et al., 2014).

All of these mechanisms have been shown to play a role in DS and might, therefore, provide suitable epigenetic targets. Genome-wide DNA hypermethylation is implicated in DS (Kerkel et al., 2010; Sailani et al., 2015) and might represent a biomarker for DS-related neurodegeneration (Sanchez-Mut et al., 2016); DNA methylation signatures are associated with premature aging and negative neurodevelopmental effects in DS (Bacalini et al., 2015; Horvath et al., 2015; Lu et al., 2016). Further, 5-methyl-cytosine and 5-hydroxymethyl-cytosine are implicated in CpG island methylation in DS (Mendioroz et al., 2015). In terms of specific targets, *DNMT3L* is found on HSA21, and its downstream effectors DNMT3a and 3b are DNA methylators that might account for the hypermethylated phenotype and cognitive deficits. Although the causal mechanism for this is uncertain, DNA methylation levels are positively correlated with cognitive function in DS, as measured by the Dalton Brief Praxis test (Jones et al., 2013). Other DNA methylation targets include the methyl donor as S-adenosyl methionine (SAM), which is present at reduced levels in DS due to overexpression of cystathionine beta-synthase (CBS) (Infantino et al., 2011). Instead of resulting in hypermethylation, CBS overexpression, and consequent SAM deficiency are thought to lead to mitochondrial dysfunction via hypomethylation of mitochondrial DNA (Infantino et al., 2011; Dekker et al., 2014).

Histone modifications represent a major type of epigenetic alteration that alters gene expression by modifying chromatin structure. Histone methylation is usually associated with transcriptional repression (some notable exceptions being methylation of lysine 4 on H3 and arginine residues on H3 and H4, which result in transcriptional activation), so histone demethylase inhibitors have been used both experimentally and clinically to reverse histone demethylation for transcriptional repression (Huang et al., 2007). Although the post-translational histone landscape has yet to be systematically characterized in DS, the DSCR (Down syndrome critical region; a genomic region shared by individuals with a given phenotype) contains the gene *DYRK1A*, whose downstream targets include direct phosphorylation of the SIRT1 histone deacetylase and the cyclic AMP response element-binding protein (CREB) and indirect regulation of gene expression via neuron-restrictive silencer factor (NRSF) (Dekker et al., 2014). The former may deteriorate cognitive function by promoting deacetylation of histone tails or recruiting the CBP/P300 histone acetyltransferase to promote CREB-related gene expression (Weeber and Sweatt, 2002). NSRF expression is decreased in DS and has been shown to be a "master regulator" of neurogenesis (Schoenherr and Anderson, 1995).

Although less is known about the function and role of histone core variants and chromatin proteins in neurobiology and DS, in particular, histone core variant pseudogenes are expressed on chromosome 21 (*H2AFZP* and *H2BFS*) and might, therefore, be worthy of further investigation (Sanchez-Mut et al., 2016). Further, *CHAF1B* and *HMGN1*, which encode two constitutive histone proteins, are found in the DSCR and recruit H3 and H4 (Kaufman et al., 1995) and regulate the expression of MeCP2 (Abuhatzira et al., 2011; Mcgraw et al., 2011), a learning disability-related protein, respectively.

Finally, various small noncoding RNAs are found on HSA21 and might therefore contribute to DS pathogenesis (miRNA-99a, miRNA-125b-2, miRNA-155, miRNA-802, and let-7c) (Sanchez-Mut et al., 2016; Sethupathy et al., 2007), with some of these implicated in neurodevelopmental diseases such as miRNA-802 in Rett syndrome, which again targets MeCP2 (Samaco and Neul, 2011; Sanchez-Mut et al., 2016). Even less is known about the role of lncRNAs in DS, but several are known to reside on HSA21 (Bhartiya et al., 2013).

3. Epigenetic targets in Down syndrome: indicators from pre-clinical studies

The preceding discussion, therefore, identifies a number of epigenetic molecules and pathways that might be therapeutically targeted to treat the cognitive defects seen in DS. Given that epigenetic drugs – *"epidrugs"* – are already in clinical use for psychiatric (e.g., valproic acid) or neoplastic diseases (e.g., decitabine, vorinostat), drug repositioning may be viable for individuals with DS (Dekker et al., 2014). Epigenetic targets are particularly attractive since – in contrast to removing or silencing an entire aneuploid chromosome – epigenetic marks are reversible.

Although trial results have been variable, there is clinical precedent for using epidrugs in humans, particularly DNA