Down syndrome and microRNAs (Review)

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Abstract. In recent years numerous studies have indicated the importance of microRNAs (miRNA/miRs) in human pathology. Down syndrome (DS) is the most prevalent survivable chromosomal disorder and is attributed to trisomy 21 and the subsequent alteration of the dosage of genes located on this chromosome. A number of miRNAs are overexpressed in down syndrome, including miR-155, miR-802, miR- 125b-2, let-7c and miR-99a. This overexpression may contribute to the neuropathology, congenital heart defects, leukemia and low rate of solid tumor development observed in patients with DS. MiRNAs located on other chromosomes and with associated target genes on or off chromosome 21 may also be involved in the DS phenotype. In the present review, an overview of miRNAs and the haploinsufficiency and protein translation of specific miRNA targets in DS are discussed. This aimed to aid understanding of the pathogenesis of DS, and may contribute to the development of novel strategies for the prevention and treatment of the pathologies of DS.

Contents

1. Introduction

Down syndrome (DS) is the most prevalent chromosomal disorder with an incidence rate between 1 in 1,000 to 1 in 1,100 live births worldwide (1). The incidence rate is higher in mothers of >35-years-old and increases with further advances in maternal age (2). DS was clinically described by John Langdon Down in 1866, prior to the identification of the genetic basis of the syndrome (3). Subsequently, an additional chromosome of the later termed chromosome 21, as the cause of DS, was discovered in 1959 by Lejeune *et al* (4). It is reported that ~95% of patients with DS have this type of trisomy, and that ~4% of children with DS exhibit trisomy 21 due to translocation between chromosome 21 and most often an acrocentric chromosome (5). Additionally, ~1% of patients are mosaic, and exhibit somatic cells with normal karyotype alongside others with trisomy (5). These patients typically present with a less serious phenotype. Rarely, there is partial trisomy 21, characterized by triplication of only part of chromosome 21 (6). This can aid to determine the regions of chromosome 21 that are key contributors to the DS phenotype. Regarding DS phenotype, it has been reported that the D21S55 region on proximal 21q22.3 contains genes that, when overexpressed, serve major roles in the pathogenesis of DS (7). However, genes outside this region also contribute to DS phenotypes (8). Furthermore, the etiology of the DS phenotype is complex and includes other mechanisms, including epigenetic pathways (9). To date, there has been no pathogenetic model linking specific structural and functional aspects of chromosome 21 to the DS phenotype. Nonetheless, in recent years, the involvement of non-coding RNAs, including microRNAs (miRNA/miRs), and epigenetic mechanisms have been linked to the DS phenotype, particularly to the intellectual disability associated with DS.

miRNAs are endogenous RNAs of ~23 nucleotides in length that pair with the mRNAs of protein-coding genes to direct post-transcriptional repression, and thus serve important roles in regulating genes in eukaryotes (10). Notably, a range of cellular processes, including cell proliferation, apoptosis and tumorigenesis, organogenesis, hematopoiesis and developmental timing, are controlled by miRNAs (11).

In the present review, the possible involvement of multiple miRNAs in the development of the DS phenotype, characterized by mental retardation, congenital heart defects, leukemia, the absence of cardiovascular disease and a low rate of solid tumor development, is summarized.

2. Down syndrome

DS is a gene dosage disorder caused by increased production of the gene products of chromosome 21. For instance, in DS, the

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triplicate copy of superoxide dismutase 1 (SOD1) gene, located on 21q22.11, is responsible for overproduction of SOD1, which leads to the oxidative stress observed in DS patients (12). This oxidative stress may manifest as multiple characteristics of the DS phenotype, including as cataractogenesis (12) and premature aging (13). Variable mental retardation occurs in all patients, and the malformative features of the phenotype are also variable (3). Generally patients with DS are underweight and exhibit delayed growth, severe hypotonia and several dysmorphic features (8). The latter includes brachycephaly and plagiocephaly, upslanting palpebral fissures, epicanthus, low-set ears, tongue protrusion, short hands, single transverse palmar crease and clinodactyly (14). Individuals with DS develop a high frequency of infections due to immunological and non-immunological factors. Among the immunological factors are suboptimal antibody responses and poor cellular chemotaxis (15), while the non-immune factors include airway anomalies, gastro-oesophageal reflux and ear anomalies (15). Additionally, ~40% of patients exhibit congenital heart defects (16).

DS patients also have transient leukemoid reactions (17) and an increased risk of developing leukemia, most commonly megakaryoblastic (M7) leukemia (18). This may be due to somatic mutations of the X-chromosomal gene encoding GATA-binding protein 1 (GATA1), an important transcriptional regulator of normal megakaryocytic differentiation, which have generally been identified in DS leukemic cells (19).

Notably, the frequency of solid tumors in DS, namely neuroblastomas and nephroblastomas in infants and common epithelial tumors in adults, is reduced compared with normal populations (20). The involvement of two genes has been implicated in this lower tumor incidence in individuals with trisomy 21, namely ETS proto-oncogene 2 (ETS2) (21) and DS candidate region-1 (DSCR1), with the latter encoding a protein that is able to suppress tumor growth in mice (22). DSCR1 also regulates the calcineurin pathway to suppress vascular endothelial growth factor-mediated angiogenic signalling (22).

Patients with DS develop premature dementia and have an increased risk of Alzheimer's disease (23), probably due to the roles of amyloid precursor protein (APP) gene, located on 21q21.3, in DS and AD. Indeed, partial trisomy 21 without triplication of the APP gene does not lead to AD (24). Furthermore, compared with healthy individuals, significantly lower systolic and diastolic blood pressures and absence of atherosclerosis are reported in DS patients (25,26). This protection against atherosclerosis may be due to the reduced level of heart-type fatty acid binding protein (27). A number of DS patients also present gastrointestinal disorders, namely duodenal stenosis, gastroesophageal reflux, imperforate anus and Hirschsprung's disease (28). Hypothyroidism also frequently develops in DS patients (29). Collectively these reports demonstrate the complexity and distinct characteristics of the DS phenotype.

3. miRNAs

Primary miRNA transcripts (pri-miRNAs) that contain cap structures and poly(A) tails are generated by RNA polymerase II, which transcribes miRNA genes (30). The maturation of pri-miRNAs occurs by two main events: i) Processing of the pri-miRNAs into stem-loop precursors of ~70 nucleotides (pre-miRNAs) in the nucleus, and ii) processing of pre-miRNAs into mature miRNAs in the cytoplasm (31). The initiation step of miRNA processing in the nucleus is cleavage by the RNase III, human Drosha (32). This nuclease is a component of two multi-protein complexes: A larger complex containing multiple classes of RNA-associated proteins and a smaller complex composed of Drosha and the double-stranded-RNA-binding protein DGCR8, the product of the DiGeorge syndrome critical region gene 8 (33). Exportin-5 mediates the nuclear export of pre-miRNAs and binds processed pre-miRNAs in a Ran guanosine triphosphate-dependent manner (34). In the cytoplasm, the pre-RNA is processed by Dicer, generating an miRNA of ~22 nucleotides long (35). These miRNA sequences are incorporated into the RNA-induced silence complex (RISC) that targets mRNAs for degradation (35). However, only a single strand of the miRNA duplex remains in the RISC complex to control the expression of target genes (36).

miRNAs bind to the 3´-untranslated region (3´-UTR) of target mRNAs to suppress their expression. Interactions among factors associated with the 3´UTR of the target mRNA, including translation regulators, RISC and mRNA decay factors, may determine the trigger event of miRNA-mediated gene silencing (37). Due to the limited complementarity between miRNAs and their targets, there are hundreds of potential mRNA targets per miRNA (38). Thus, a single miRNA may regulate multiple biological processes (39), and several miRNAs can regulate an individual target (38). Additionally, miRNA expression varies depending on cell type and cellular conditions (38). The implications of miRNAs in the pathogenesis of DS are subsequently addressed.

4. Down syndrome and chromosome 21 miRNAs

The miRBase (http://www.mirbase.org/search.shtml, accessed on 4/9/2017) indicates the presence of 29 Homo sapiens miRNAs on chromosome 21 (Fig. 1). However, only some of these miRNAs have been identified to have transcription levels at the expected 1.5 ratio due to chromosome 21 trisomy in DS (40). Notably, five of these miRNAs that meet the overexpression ratio, namely miR-155, miR-802, miR- 125b-2, let-7c and miR-99a, have been implicated to be involved in DS (41,42). In turn, the specific target genes of these miRNAs are haploinsufficient in DS (43,44). For instance, miR-155 targets complement factor H mRNA (CFH), which is decreased in DS tissues (45). As CFH protects neurons from axonal injury, complement opsonization, and leukocyte infiltration in the brain parenchyma (46), overexpression of miR-155 may be involved in the brain pathology of DS patients. Additionally, CFH is a repressor of the immune response (45). Thus, among other known factors described above (15), this repression may be a cause of susceptibility to infection in DS patients.

In T21 induced pluripotent stem neuronal progenitor cells (iPS-NPCs), Lu *et al* (47) observed the degradation of methyl-CpG binding protein 2 (MeCP2) following overexpression of miR-155 and miR-802. Additionally, they observed that T21 iPS-NPCs exhibited developmental defects and generated fewer neurons than controls (47). Decreased MeCP2 may also contribute to the neurochemical abnormalities observed in the brains of DS individuals (43). In the study of

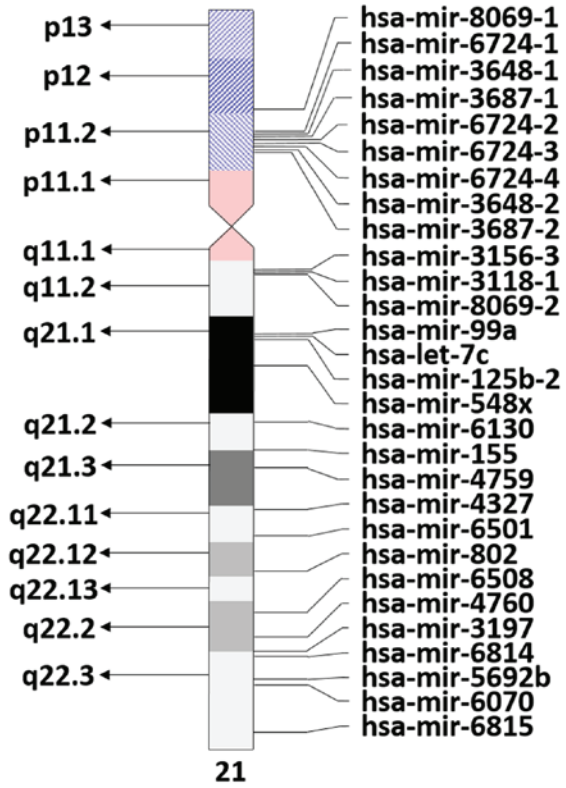

Figure 1. miRNA loci on chromosome 21. mir/miRNA, microRNA; hsa, Homo sapiens.

hippocampal neurons from mice that either lacked expression or expressed twice the normal levels of MeCP2, Chao *et al* (48) identified that the regulation of glutamatergic synapse number by MeCP2 may be a mechanism for the altered synaptic strength of neurons in DS. Keck-Wherley *et al* (49) reported that miR-155 and miR-802 were significantly increased in the DS mouse model Ts65Dn, and that significant overexpression of these miRNAs may be implicated in hippocampal deficits in DS phenotypes. Indeed, the hippocampus is an important region involved in learning and memory and in long-term synaptic plasticity.

Underexpression of angiotensin II type 1 receptor, a target of miR-155, may explain the absence of cardiovascular disease in DS individuals (43), as this receptor has been implicated in cardiovascular pathologies (50). Additionally, Coppola *et al* (51) observed overexpression of the miR-99a/let-7c cluster and subsequent decrease of their targets in fetal DS heart tissue, suggesting that the cluster may contribute to congenital heart defects in DS.

miR-125b-2 may serve a role in the regulation of megakaryopoiesis and may be an oncogenic miRNA involved in the pathogenesis of megakaryoblastic leukemia observed in DS (52). Zhang *et al* (53) demonstrated that the most expressed miRNAs in pediatric acute myeloid leukemia (AML) were miR-100, miR-125b, miR-335, miR-146, and miR-99a. MiR-155 was also identified to be elevated in the bone marrow of some patients with AML (54). Furthermore, leukemic cells from DS patients with acute megakaryoblastic leukemia have been demonstrated to contain acquired mutations in GATA1, which serves as an important hematopoietic transcription factor (55). Shaham *et al* (56) also documented a cooperation between GATA1 and miR-486-5p and observed that miR-486-5p enhanced the survival of leukemic cells from DS patients.

Overexpression of miR-125a or miR-125b in an Erb-B2 receptor tyrosine kinase 2-dependent human breast cancer cell line impaired its growth potential and reduced its motility and invasive capabilities (57). Overexpression of the miRNA let-7 has also been identified in breast cancer, and may regulate the tumorigenicity of breast cancer cells (58). In particular, let-7c inhibited the tumor formation capacity of breast cancer stem cells (59). These results may explain the low rate of breast cancer among women with DS.

Furthermore, Johnson *et al* (60) demonstrated that let-7 was highly expressed in lung tissue, repressed cell proliferation in lung cells and affected cell cycle progression in a liver cancer cell line. They also identified that let-7 regulated cell cycle-related genes involved in the repression of cell proliferation pathways (60). In particular, let-7c may inhibit lung adenocarcinoma proliferation (61).

Prostate cancer is also less common in patients with DS compared with healthy individuals (62). The miR-99 family of miRNAs have been reported to inhibit the proliferation of prostate cancer cells and decrease the expression of prostate-specific antigen, a biomarker for prostate cancer diagnosis (63).

Collectively these reports may explain the low risk of solid tumor development in patients with DS (64). However, other factors have been suggested to explain this low tumor risk, including high expression of the calcineurin inhibitor DSCR1 on the basis of its inhibition of vascular endothelial growth factor-mediated angiogenic signalling (22).

A previous study identified two novel miRNAs, miR-nov1 and miR-nov2, on chromosome 21, located up- and downstream of the annotated miR-802 loci (40). miR-nov2 is located in the 'DS critical region' (chr21q22.2) and its overexpression has been identified in DS lymphocytes (40). Xu *et al* (40) predicted that the 97 mRNA targets of miR-nov2 were associated with cell growth, cell death, cellular localization and protein transport. In a subsequent study, they also confirmed the identification of miR-nov1 and miRnov2 in cord blood mononuclear cells of DS fetuses (65). Notably, it was observed that miR-99a, let-7c, miR-125b-2 and miR-155 were downregulated in DS cells (65). Thus, the role of these miRNAs in the development of the DS phenotype should be investigated.

5. miRNAs derived from other chromosomes associated with DS phenotype

Using microarray technology to identify miRNAs that were aberrantly expressed, Lim *et al* (66) compared genome-wide miRNA expression in the placentas of normal and DS fetuses. They observed that no chromosome 21-derived miRNAs were differentially expressed. However, of the 584 genes on chromosome 21, 76 were differentially expressed and possible targets of miRNAs. These target genes on chromosome 21 were significantly associated with DS phenotypes, including mental retardation and congenital abnormalities (66). Nevertheless, the absence of differentially expressed miRNAs on chromosome 21 between DS and normal placentas disagrees with other studies conducted in fetal cord blood cells (65). Lim *et al* (66) proposed that this variance may be due to differences in the

characteristics of tissues reported in previous studies. Indeed, Liang *et al* (67) identified that numerous miRNAs had distinct expression in the placenta compared with other tissues.

However, the results of Lim *et al* are also contrasting to the results of Svobodová *et al* (68), who observed that three miRNAs located on chromosome 21 (miR-99a, miR-125b and let-7c) and four miRNAs located on other chromosomes (miR-542-5p, miR-10b, miR-615 and miR-654) were upregulated in DS placentas. Additionally, Lim *et al* (69) reported that miRNA expression was significantly different between blood and placenta samples, and that mir-1973 and mir-3196 were overexpressed in the trisomy 21 placenta. These two miRNAs may regulate target genes involved in development of the nervous system (69).

Shi *et al* (70) studied the microRNA expression profile of hippocampal tissues from DS fetuses using miRNA microarray, and reported that the function of miR-138-5p and the downregulation of its target, enhancer of zeste homolog 2, in the hippocampus may be involved in the intellectual disability of DS patients. Furthermore, Wang *et al* (71) reported that interleukin (IL)-1β, IL-12 receptor subunit β2, autism susceptibility candidate 2 (AUTS2) and KIAA2022 may be involved in atrioventricular septal defect in DS patients, and that AUTS2 and KIAA2022 may be targeted by miR-518a, miR-518e, miR-518f, miR-528a and miR-96.

Lin *et al* (72) studied the expression profiles of miRNA and protein in cord blood samples from DS and normal fetuses, and reported that three miRNAs (miR-329, miR-27b and miR-27a) and seven proteins (growth factor receptor-bound protein 2, thymosin β10, RuvB-like 2, mitogen-activated protein kinase 1, tyrosine-protein phosphatase non-receptor type 11, α-actin-2 and protein tyrosine kinase 2) exhibited high levels of differential expression in DS fetuses. This differential expression may serve a role in the pathogenesis of DS. More recently, Arena *et al* (73) reported a higher level of miR-146a expression in astroglial cells within the hippocampal white matter of DS fetuses compared with normal fetuses, and identified persistence of this elevated expression postnatally. This may be a key finding, as the expression level of miR-146a has been suggested as an important determinant for neuronal development (74).

Thus, the study of these miRNAs in DS cells may contribute towards greater comprehension of the DS phenotype.

6. Conclusion

The overexpression of several miRNAs, including miR-155, miR-802, miR-99 and let-7c, and the consequent haploinsufficiency of their specific target proteins are potentially involved in the DS phenotype. In particular, miR-155 and miR-802 may be involved in neuropathology, the cluster miR-99/let-7c in congenital heart defects and miR-155 in the absence of cardiovascular disease observed in DS. Additionally, miR-125b-2, miR-155 and miR-99a possibly serve roles in the pathogenesis of megakaryoblastic leukemia in DS patients. A number of miRNAs expressed in DS patients may also be implicated in the low rate of solid tumor development in DS patients, including miR-125b and let-7c in breast cancer, miR-99 in prostate cancer and let-7c in lung cancer. Nevertheless, the role of miRNAs located on other chromosomes, and with target genes are on or off chromosome 21, should not be excluded from the DS phenotype.

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