Annals of Diagnostic Pathology 34 (2018) 103–109

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

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

microRNA 155 up regulation in the CNS is strongly correlated to Down's syndrome dementia☆

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ARTICLE INFO

Keywords:
Dementia
Down's syndrome
microRNA
Bcl2 family
Tau

ABSTRACT

This study examined the molecular correlates of Down's dementia. qRTPCR for chromosome 21 microRNAs was correlated with in situ hybridization, immunohistochemistry for microRNA targets, mRNAs located on chromosome 21, and neurofibrillary tangles in human and the Ts65 dn mouse Down's model. qRTPCR for the microRNAs on the triplicated chromosome showed miR-155 dominance in brain tissues (14.3 fold increase, human and 24.2 fold increase, mouse model) that co-expressed with hyperphosphorylated tau protein. miR-155 was not elevated in Alzheimer's disease or neonates with Downs' syndrome. Chromosome 21 genes APP/BA-42, DYRK1a and BACH1 were not correlated to pathologic changes in Down's dementia. Validated CNS targets of miR-155 that were present in controls and Alzheimer's disease but lacking in Down's dementia brains included BACH1, CoREST1, bcl6, BIM, bcl10, cyclin D, and SAPK4. It is concluded that Down's dementia strongly correlated with overexpression of chromosome 21 microRNA 155 with concomitant reduction of multiple CNS-functional targets. This study highlights the need for anatomic pathologists to determine the specific and diverse pathways cells may take to form neurofibrillary tangles in the different dementias.

1. Introduction

By age 40 most people with Down's syndrome have dementia [1-3]. The brains of most adults with Down's syndrome show plaque deposits and neurofibrillary tangles, both hallmarks of Alzheimer's disease [1-3]. Several genes implicated in the Alzheimer's disease are present on chromosome 21 that is triplicated in Down's syndrome: amyloid beta precursor protein (APP), superoxide dismutase (SOD), BACH1, and dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1a) [4-7].

APP breakdown can produce amyloid beta peptide precipitates that form senile plaques in the brain. The extra copy of the SOD gene in Down's syndrome could lead to the accumulation of toxic superoxide radicals; it has been shown that overexpression of SOD results in learning and memory deficiencies [4]. BACH1 is a translational repressor that can inhibit the cell's response to oxidative stress and has also been linked to neurologic symptoms in Down's syndrome [6]. DYRK1a is involved in the phosphorylation of tau protein and has been implicated in neurofibrillary tangles [5,7]. It has been shown that increased expression of DYRK1a in animal models induces brain dysfunction due to increased progression of progenitor cells to differentiated neurons [5,7].

MicroRNAs are key regulatory molecules in neural development, given their ability to disrupt expression of target mRNAs. Chromosome 21 has several microRNAs that include a cluster (microRNA-let7c, 99a, and 125b), miR-155, and miR-802 that have been implicated in brain function [1,8-17]. Inhibition of miR-155 increases survival in a mouse model of ALS [12]. miR-155 and -802 are increased in the hippocampus of the brain in the Ts65Dn mouse model of Down's dementia [17]. Both miR-let-7c and -125b have been shown to enhance neuronal aging and degeneration [15]. miRNA dysregulation on chromosome 21 in Down's syndrome is tissue dependent; miR-155 is increased in the brain and the white blood cells in the Ts 65 dn model of Down's syndrome but is not detected in the placenta or lung [17]. Similarly, of the chromosome 21

☆ Financial support: Alzheimer's disease Drug Discovery Foundation #20160204.
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https://doi.org/10.1016/j.anndiagpath.2018.03.006

miRNAs, only miR-802 is increased in fetuses with Down's syndrome but none are increased in the placenta [18,19].

The purpose of this paper is to analyze the molecular correlates of Down's dementia as it relates to chromosome 21 microRNA and gene expression, and to compare this with Alzheimer's disease.

2. Materials and methods

2.1. Tissues

Formalin fixed, paraffin embedded tissues were available from the files of Folio (Alzheimer's disease, controls) and from consult files (GJN, Down's syndrome). The age, specific brain region of the tissue, and BRAAK scoring were available. For Alzheimer's disease, 15 formalin-fixed, paraffin embedded tissues samples from 8 separate donors that included cortex and hippocampus were available from people who had BRAAK scores of IV to VI (mean age 75.4). Fifteen formalin fixed, paraffin embedded tissues sections from age matched controls served as negative controls. For Down's disease, 12 tissues were available from 3 donors who died with dementia and ranged in age from 34 to 42 years old. Ten aged matched controls were also studied. Eight brain tissues from neonates who died in utero from Down's syndrome were studied, each was from the cortex or hippocampus with 5 neonatal matched controls.

2.2. N1E-115 transfection experiments

The murine neuroblastoma cell line N1E -115 (ATCC CRL-2263) was obtained from the ATCC. The cells were grown to 50% confluence and transfected with miR-125b sense, miR-155 sense, miR-125b sense plus miR-155 sense, or sham transfected with a scrambled digoxigenin tagged LNA modified oligomer as previously described [20]. The cells were grown for 48 h after transfection and fixed in 10% neutral buffered formalin for in situ hybridization/immunohistochemistry.

2.3. microRNA isolation

Tissue samples (150 μm) of formalin fixed, paraffin embedded tissues that were adjacent to sections shown to be strongly positive for hyperphosphorylated tau protein were deparaffinized and then stored in RNAlater®-ICE (*Ambion*, AM7030) at −20 °C for a minimum of 16 h and the RNA extracted via a standard published protocol using the RNA/DNA/Protein Purification Plus Kit (*Norgen Biotek*, #47700) [20,21].

2.4. qRTPCR

10 ng of RNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription kit (Life Technologies) with TaqMan RT primers specific for mature miRNA using a standard, published protocol (Applied Biosystems) [21].

The Comparative real-time PCR was performed in triplicate, including no-template controls and analyzed using QuantStudio 12K Flex Real-Time PCR System. The Ct Average of each triplicate was used to perform the relative quantification analysis. RNA input was normalized using RNU48 (001006) and the relative expression was calculated using the comparative Ct method.

2.5. Ts65 Dn mouse model of Down's syndrome

Brain sections of Ts65dn mice (JAX stock #001924) were studied. These mice have three copies of most of the genes on mouse chromosome 16 that is equivalent to human chromosome 21. Although they do have three copies of miR-155 and -802, miR-let-7c, -99a, and -125b are not triplicated [17].

2.6. In situ hybridization

The microRNA in situ hybridization protocol has been previously published [20,21]. In brief, in situ hybridization for miR-let-7c, -99a, -125b, -155, and -802 (each 5p) was done using LNA modified antisense oligomers that were 5′ tagged with digoxigenin (Exiqon) where the reporter chromogens nitro-blue tetrazolium and 5-bromo-4-chloro-3′-indolyphosphate yield a blue signal. The TUNEL assay was done as previously described [20,21].

2.7. Immunohistochemistry

The immunohistochemistry protocol has been previously published [20,21]. The tissues were tested for the following antigens: cyclin D (Ventana Medical Systems), hyperphosphorylated tau protein, beta-amyloid 42, amyloid beta precursor protein, Bcl6, Bcl10, Bcl2, BIM, activated caspase-3, cFLIP, DYRK1a, SAPK4, ROR1 (ABCAM), BAK, BAX, SOD, MCL1 (Enzo Life Sciences), BACH1 and CoREST1 (ProteinTech). The analyses were done on the automated Leica Bond platform with the modification that we used the Enzo Life Sciences peroxidase conjugate (catalogue # ADI-950-113-0100).

2.8. Co-expression analysis

Co-expression analyses were done using the Nuance system (CRI) as previously published [20,21]. In brief, a given tissue was tested for two different targets using fast red, NBT/BCIP or DAB as the chromogens. The results were then analyzed by the Nuance and InForm systems to determine what percentage of cells were expressing the two targets of interest.

2.9. Statistical analyses

Statistical analysis was done using the InStat Statistical Analysis Software (version 3.36) and a paired *t*-test (also referred to as a "repeated measure t-test"). The null hypothesis was rejected if the significance level was below 5%.

3. Results

3.1. Distribution of β amyloid-42, and hyperphosphorylated tau protein in Down's dementia

β amyloid-42 positive plaques were detected in 14/15 (93%) of the Alzheimer's disease tissues and 2/15 (13%) of the controls. The senile plaques were widely distributed and showed the typical central precipitate (Fig. 1). Senile plaques were noted in 3/12 (25%) of the Down's dementia cases (Fig. 1); 0/10 of the aged matched Down's dementia controls showed senile plaques.

Hyperphosphorylated tau protein defined neurofibrillary tangles were evident in 13/15 (87%) of the Alzheimer's disease tissues, 11/12 (92%) of the Down's dementia tissues, and 0/25 of the controls (Fig. 1). The hyperphosphorylated tau protein signal was in neurons as defined by co-expression with pyruvate dehydrogenase and NeuN (data not shown). Neither B amyloid-42 or hyperphosphorylated tau protein was evident in the 8 brain tissues from neonates who died in utero with Down's syndrome (Fig. 1).

3.2. Detection of microRNAs let-7c, -99a, -125b, -155, and -802 in the brain tissues from people with Down's syndrome

Each of the controls, Alzheimer's disease tissues, and fetal Down's syndrome brain tissues were negative for these chromosome 21 microRNAs as defined by < 1% of cells with a signal (Fig. 2). miR-let-7c, -99a, -125b, and -155 were evident in 11/12 (92%) of the Down's dementia tissues and were highly expressed as from 13 to 58% of