Free Radical Biology and Medicine (xxxx) xxxx–xxxx

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

4-hydroxynonenal protein adducts: Key mediator in Rett syndrome oxinflammation

Giuseppe Valacchi[a,b,*,1], Alessandra Pecorelli[a,b,1], Carlo Cervellati[c], Joussef Hayek[d]

[a] Plants for Human Health Institute, Department of Animal Sciences, NC State University, NC Research Campus, 600 Laureate Way, Kannapolis, NC 28081, USA
[b] Department of Life Sciences and Biotechnology, University of Ferrara, Via Luigi Borsari 46, 44121 Ferrara, Italy
[c] Department of Biomedical and Specialist Surgical Sciences, Section of Medical Biochemistry, Molecular Biology and Genetics, University of Ferrara, Via Luigi Borsari 46, 44121 Ferrara, Italy
[d] Child Neuropsychiatry Unit, University Hospital, AOUS, Viale Mario Bracci, 53100 Siena, Italy

ARTICLE INFO

Keywords:
Inflammation
Isoprostanes
Oxidative stress
Rare disease
Orphan disease
CDKL5
FOXG1
MecP2

ABSTRACT

In the last 15 years a strong correlation between oxidative stress (OxS) and Rett syndrome (RTT), a rare neurodevelopmental disorder known to be caused in 95% of the cases, by a mutation in the methyl-CpG-binding protein 2 (*MECP2*) gene, has been well documented. Here, we revised, summarized and discussed the current knowledge on the role of lipid peroxidation byproducts, with special emphasis on 4-hydroxynonenal (4HNE), in RTT pathophysiology. The posttranslational modifications of proteins via 4HNE, known as 4HNE protein adducts (4NHE-PAs), causing detrimental effects on protein functions, appear to contribute to the clinical severity of the syndrome, since their levels increase significantly during the subsequent 4 clinical stages, reaching the maximum degree at stage 4, represented by a late motor deterioration. In addition, 4HNE-PA are only partially removed due to the compromised functionality of the proteasome activity, contributing therefore to the cellular damage in RTT. All this will lead to a characteristic subclinical inflammation, defined "OxInflammation", derived by a positive feedback loop between OxS byproducts and inflammatory mediators that in a long run further aggravates the clinical features of RTT patients. Therefore, in a pathology completely orphan of any therapy, aiming 4HNE as a therapeutic target could represent a coadjuvant treatment with some beneficial impact in these patients.

1. Introduction

1.1. Rett syndrome

Rett syndrome (RTT) is a rare and debilitating neurological disorder that affects approximately one in every 10,000/15,000 females and is only rarely observed in males [1]. A typical aspect of RTT is a phase of normal development for 6–18 months after birth, followed by a stagnation and a delayed onset of symptoms with progressive loss of milestones and cognitive disability. In particular, the characteristic disease progression, which evolved in four clinical stages, leads to a myriad of typical signs, including overall growth retardation, loss of speech and motor functions, such as loss of hand skills and development of stereotypical hand movements. In addition, affected patients typically show social interaction deficits as well as autism-like traits; besides, some features such as microcephaly, breathing irregularities and apneas, heart problems, gastrointestinal abnormalities, seizures, scoliosis, abnormal sleep patterns and early hypotonia are also common [2].

Most cases of "classic" or "typical" RTT (> 96%) are caused by *de novo* mutations in the X-linked gene *MECP2* and arise in germ cells, usually of the paternal origin [3,4]. Due on the type, location and severity of the genetic mutations and balance of X-inactivation, RTT shows a wide range of phenotypic outcomes, ranging from mild to extremely severe clinical presentations [5,6]. Loss-of-function mutations in *MECP2* are also responsible for several other conditions, included in the MECP2 Spectrum Disorders, such as severe neonatal encephalopathy, bipolar disorder, schizophrenia, Angelman-like syndrome, mental retardation, and autism [7–9]. According to the new revised criteria [10], patients with nonclassic phenotypes are considered to have "variant" or "atypical" forms of RTT, including: "Preserved Speech Variant" (PSV), also caused by *MECP2* mutation and characterized by milder clinical abnormalities and by the appearance of some degree of speech [11]; "Early Seizure Variant" and "Congenital

* Correspondence to: Dept. of Animal Science, North Carolina State University, Plants for Human Health Institute, NC Research Center, 28081 Kannapolis, NC, USA.
E-mail addresses: giuseppe.valacchi@unife.it, gvalacc@ncsu.edu (G. Valacchi).
[1] Equally contributed.

http://dx.doi.org/10.1016/j.freeradbiomed.2016.12.045
Received 14 December 2016; Received in revised form 24 December 2016; Accepted 30 December 2016

Variant", known to be caused by mutations in other *loci*, i.e. *CDKL5* and *FOXG1*, and characterized by unique features, as early infantile seizures and congenital onset [12,13]. Among RTT atypical forms, for their specific gene mutations and peculiar clinical features, the two variants *CDKL5* and *FOXG1* are hence considered as distinct clinical and molecular entities [14].

The epigenetic factor MeCP2 binds to methylated cytosines in target gene promoters and, interacting with other co-factors, is involved in the regulation of their transcription as both repressor or activator [15–17]. More recent evidence shows that MeCP2 can also influence alternative splicing of downstream gene products, expression of various micro-RNAs and long non-coding RNAs as well as act like a chromatin-remodeling protein, triggering the chromatin compaction at methylated DNA sites with consequent regulation of the transcription of adjacent genes [18]. Although the protein is ubiquitously transcribed among various tissues, its levels appear higher in the brain, in particular in neurons, where it plays an essential role not only in neuronal maturation, dendritic arborization and synaptogenesis, but also in maintaining mature neuronal networks and electrophysiological responses throughout life [19–22].

In addition, recent studies have also reported the presence of MeCP2 in all glial cell types including astrocytes, oligodendrocyte progenitor cells and oligodendrocytes [23–27] and, to date, it is well clear that MeCP2 deficiency in both neurons and glia can have a profound impact on brain function and contribute to manifestation of specific RTT symptoms [21,28], while the restoration of Mecp2 in astrocytes can improve some of these manifestations including locomotion defects, anxiety levels, and respiratory abnormalities, greatly prolonging lifespan in Mecp2-deficient mouse models [25,26,29,30].

The wide variety of MeCP2 functions clearly points out how complex can be the range of possible mechanisms leading from the gene mutations to the RTT phenotypes. In light of the recent insights, a new scenario is emerging in which neurons start and direct the pathology in connection with the dysfunction of other cell types and peripheral tissues which, exacerbating some symptoms, can contribute to further amplify neurologic problems, ultimately, accomplishing a positive feedback loop. However, while the structure, functions, interactions and expression of MeCP2 have been widely explored and a number of their aspects has been revealed, much about this protein with pleiotropic actions remains yet to be discovered.

1.2. Evidences of redox imbalance in RTT

Compelling evidences point out the possible role of oxidative stress (OxS) as a player in the pathogenic mechanisms of RTT [31–33]. Based on a number of reports, the redox imbalance is a peculiar condition that occurs in both RTT patients and RTT animal models [34,35]. A close relationship between the different clinical stages/phenotypes of the syndrome and the OxS levels has been well recognized in RTT patients [36–39]. In RTT mice, the increased brain oxidative damage appears as an event that precedes the onset of the hallmark features of the disease, suggesting that Mecp2 protein deficiency and aberrant redox homeostasis could be inextricably linked to each other [35].

Disturbances of the normal redox status in RTT can arise from either oxidants overproduction and impaired antioxidant defense system. While the precise cascade of the molecular events linking the *MECP2* mutation to the oxidative imbalance remain yet little decipherable, now there are increasing evidences that mitochondrion can represent the initial source of oxidants production in RTT, as consequence of its dysfunction [32,40–43]. In addition to mitochondria, also NADPH oxidase (NOX) activation appears as a source of endogenous OxS, able to play a possible role in RTT patients oxidative damage (Fig. 1) [44].

A wide number of studies, both in cellular and tissue samples from murine models and patients, clearly demonstrated the mitochondrial dysfunction in RTT. Since the late 80's and also more recently, several reports showed the presence of mitochondrial ultrastructural abnormalities in biopsies of brain, liver, muscle and skin as well as in peripheral blood mononuclear cells (PBMC) from subjects affected by RTT. The mitochondrial morphological changes consisted in swollen organelles with vacuolization, granular inclusions and membranous changes [45–56]. Ultrastructural analyses of brain, muscle and microglia isolated from Mecp2-null mice identified widespread abnormal "dissolving" features of mitochondria; in particular, most mitochondria were enlarged, dysmorphic and had electron-lucent central matrices and non-parallel, disorganized cristae, confirming the results obtained in human studies [57–59].

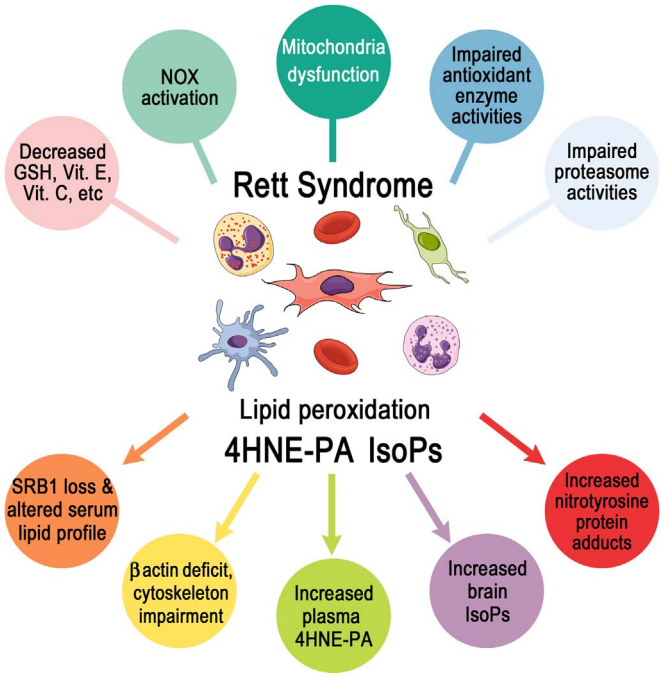

Fig. 1. Redox imbalance as a contributing factor to the pathomechanisms of Rett syndrome. Disturbances of the redox homeostasis in RTT arise from either oxidants overproduction and impaired antioxidant defence system. A wide number of studies, both in cells and tissues from patients and mouse models, clearly demonstrated, among others, mitochondrial dysfunction, NADPH oxidase (NOX) activation, low levels of GSH and other antioxidants, impaired antioxidant enzyme activities, and reduced proteasome activities. Overall, these defective molecular processes are able to cause OxS, leading to oxidative damage including increased plasma and cell levels of 4HNE-PA, loss of HDL receptor SRB1 and altered serum lipid profile, impairment of cytoskeleton proteins, increased IsoPs and nitrotyrosine protein adducts levels.

In parallel to structure abnormalities, alterations in the activity of mitochondrial respiratory chain complexes associated to impaired energy metabolism have been also detected in both RTT patients and animal models [44,52,59–68].

In addition, recent studies on gene expression profile revealed abnormalities in several genes related to mitochondrial function and/or structure in frontal cortex and PBMC from RTT patients [56,69]. Using a *Mecp2*-null mouse model, Kriaucionis et al. [62] demonstrated the overexpression of gene for ubiquinol-cytochrome c reductase core protein 1 (*Uqcrc1*), which encodes for a subunit of mitochondrial respiratory complex III, with an increase in complex III activity and a decrease in that of complex IV. Another gene for a component of complex IV, cytochrome c oxidase subunit 1 (*CO1*), was also down-regulated in the frontal cortex of RTT patients [64] and in the skeletal muscle of the symptomatic $Mecp2^{y/-}$ mice [66]. In addition, a microarray study on PBMC from RTT patients reported an up-regulation of several mitochondria-related genes. Specifically, the most significantly regulated transcripts included those encoding for several subunits of mitochondrial respiratory chain complexes and thus linked directly to mitochondrial ATP production and, indirectly, to potential oxidant generation [69]. Overall, these elements are in fully agreement