Oxidative stress: a hallmark of Rett syndrome

“As MeCP2 acts a multifunctional regulator of gene transcription, knowing the gene mutation is sufficient to make a diagnosis, but this information is not sufficient to understand the mechanisms by which, the *MECP2* gene mutation drives the clinical manifestations in this complex neurodevelopmental disease.”

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Genetic diseases associated with intellectual disability/brain damage, as well as several nongenetic neurological diseases, often share an oxidative stress (OS) mechanism [1,2]. Thus, OS could play a critical role in the neurogenesis besides other neurological regulatory mechanisms such as the γ -amino-butyric-acid-ergic neuronal function [3].

In the scientific field, OS has firstly been investigated as a mere laboratory phenomenon. Over the years, OS has been recognized by the scientific community as a major pathogenic process, linked to a disorder of the antioxidant defenses/oxidant insult balance. Its definition is not surprising as ‘stress’, given that the term stress is defined as ‘a syndrome produced by diverse nocuous agents’ whose symptoms are ‘independent of the nature of the damaging agent and represent rather a response to damage as such’ [4]. The ubiquity of OS finds a conceivable justification by considering the mechanisms of

the defense processes in living organisms. Indeed, all human conditions in which an effort is required from the organism to maintain the homeostatic equilibrium are known to lead to a hyperactivity status for inflammatory and immune cells, thus determining a free radical species stockpile. New frontiers in the involvement of OS mechanisms in pathological events are continuously explored. However, to date, human pathological conditions in which a cause–effect relationship between OS and disease is demonstrated are almost nonexistent. Nevertheless, an update on the OS mechanisms relevance for human disease becomes urgent [5]. Indeed, OS is far too often considered as a ‘secondary sign’, linked to a wide array of human diseases, with a particular focus on chronic inflammatory conditions. Therefore, OS has been mainly evaluated as a function of disease complications and/or progression, rather than as a major causative player. In this perspective, OS has been very often

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considered as a process that can accompany the disease, but is not causing the disease. Therefore, oxidative mechanisms are assumed to play a corollary or ancillary role, while almost never considered to be a hallmark of a given disease. Nevertheless, the role of free radical species in gene regulation and cellular signaling is an emerging key concept [6]. Likewise, an irreversible change in the redox status could elicit biochemical signals by inducing the cell toward apoptotic phenomena, whereas pathological conditions are the consequences of a chronic alteration in the redox homeostasis [7]. Thus, although an uncontrolled redox homeostasis might not be the main cause of human diseases, its role in the regulation of cellular mechanisms is undeniable.

Over the last few years, we found that Rett syndrome (OMIM#312750), a complex monogenic disease [8] mainly featuring an abnormal neurodevelopmental phenotype, could represent the crossroads for a unique biological triad:

- Monogenic cause with intimately related OS imbalance [9];
- Availability of synthesized OS biomarkers/bioactive molecules mediating disease phenotype expression [10];
- Availability of experimental animal models recapitulating the main features of the human disease, together with the possibility of rescuing the phenotype by acting on gene re-expression (i.e., disease reversibility) [11].

Rett syndrome is a relatively rare disorder (about 1 to 10,000 female live births), caused by a sporadic loss-of-function mutation in the methyl CpG binding (*MECP2*) gene in up to 95% of cases [12]. Nevertheless, the genetic cause is not able to elucidate the pathological events of Rett syndrome. As MeCP2 acts a multifunctional regulator of gene transcription [13], knowing the gene mutation is sufficient to make a diagnosis, but this information is not sufficient to understand the mechanisms by which, the *MECP2* gene mutation drives the clinical manifestations in this complex neurodevelopmental disease. Indeed, despite a well-established monogenic cause, Rett syndrome is a multisystemic disease, with a complex and quite heterogeneous phenotype [12]. How OS could be defined as a hallmark of Rett syndrome, such a complex genetic disease? Several data impressively indicate the central role of OS in the pathogenic mechanisms the disease: in

human Rett syndrome, signs of OS are reported in cells, including non-nervous cells, brain and blood [10,14–17]; OS status is related to the type of *MeCP2* mutations and disease clinical stage [10,17,18]; in mice models of Rett syndrome evidence for oxidative brain damage is present prior to the onset of manifestations of the disease phenotype [15]; brain oxidative homeostasis is rescued following brain-specifically *Mecp2*-reactivated mice. In particular, the complex experimental design carried out in five mouse models of the disease (*Mecp2*-null presymptomatic, symptomatic, and rescued; *Mecp2*-308 mutated presymptomatic and symptomatic) led us to infer a cause–effect relationship between oxidative brain damage and *Mecp2* loss of function. Correction of *Mecp2* deficiency appears to be able to normalize fatty acid oxidation, thus revealing that the OS imbalance is a reversible phenomenon that is sensible to the presence of a functional *Mecp2* protein [15].

Likewise, several pathological clinical manifestations in Rett syndrome appear to be significantly related to a chronic OS status, well documented during the course of disease progression. The chronic pathological manifestations can be the result both of free radicals toxicity and changes in gene expression.

In Rett syndrome, we have evaluated OS from different points of view, by examining several specific biomarkers/active mediators, in order to get a more general perspective in the redox homeostasis of the disease. Pro-oxidant agents, antioxidant defense and indicators of oxidative damage have been evaluated [9]. Overall, a redox dysregulation is evident in plasma and intracellular antioxidant molecule, together with relevant lipid peroxidation and protein oxidation phenomena. Our approach led to the identification of arachidonic, docosahexaenoic and adrenic acids as the major target fatty acids, chronically suffering under the oxidative insult. Moreover, these fatty acids have been indicated, not only as major target molecules for free radical species, but also as weak points in Rett syndrome. Indeed, by restoring the fatty acid profile, a significant reduction in the clinical severity is evidenced [10,17,18].

Our evidence for an intimate relationship between OS and Rett syndrome is not without relevant consequences. For instance, the use of human induced pluripotent stem cells has been previously suggested as a possible therapy in Rett syndrome [19]. However, induced pluripotent

stem cells are usually reprogrammed from fibroblast cells. Our recent evidence of severe OS and endoplasmic reticulum stress in cultured skin fibroblasts obtained from Rett patients [14] would potentially limit their application or, at least, be taken into full account as a biochemical marker of disease rescue during future therapeutic trials with stem cells.

The underlying possible source(s) for OS in Rett syndrome is a current matter of debate and a hot focus of research in our laboratory, as well other institutions around the world. According to some researchers, mitochondrial dysfunction could be the main source for an excessive free radical production in Rett syndrome [20,21]. The hypothesis of Rett syndrome as a mitochondrial disease has been based on supportive evidence on mitochondrial morphology alterations and reduction of specific enzyme activities [20] and is, of course, a quite plausible explanation, also given the specific role for the mitochondrial electron transport chain in reactive oxygen species production.

Nevertheless, a MeCP2 control on cellular signaling pathways must also be considered. In particular, molecules exist that are implicated in the general regulation of the redox homeostasis, such as the Nrf2/Keap1, MAPKs, NF- κ B, PKC, STAT3 and PPAR γ . At least for some of these signaling molecules, an epigenetic modulation by MeCP2 has already been demonstrated [22].

Thus, in our own opinion, it would not be

surprising if the real source for OS in Rett syndrome could actually be related to an aberrant regulation of a finely tuned redox genetic control, either through a direct or, more likely, an indirect action exerted by the MeCP2 protein.

If this would be the case, a new, hitherto unrecognized, major function needs to be added to the few already known ones for the MeCP2 protein.

Dedication

We dedicate this Editorial to the Rett girls and their families; to professional singer Matteo Setti for his continued interest in our research and generosity in performing charity concerts in support of our studies; and in memoriam of Professor Mario Comporti (Siena, 1935–2014), an international pioneer in the exploration of oxidative stress in disease.

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