Cell miRNAs molecular pathway: the role of EMFs

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Summary—The project is related to the possible investigation of the etiopathogenetic aspects involved in onset and evolution of Alzheimer’s Disease (AD) and their interactions with patient-specific conditions and environmental factors. In particular we will verify the effects of exposure to electromagnetic fields on AD biology and on disease progression both at cellular level (in-vitro) and at systemic level (modifications on patients’ circulating cells), focusing on microRNA dysfunction that holds promise for the diagnosis, prognosis and management of AD.

I. INTRODUCTION

Alzheimer’s disease (AD) is the most common cause of sporadic dementia; it is defined by a progressive loss of cognitive functions, ultimately leading to death [1]. The multifactorial etiology of AD remains debated, as the presence of the up to now known risk factors is not sufficient to predict the onset and the severity of the pathology [2]. The role of the post-transcriptional mechanisms in the modulation of gene expression has recently emerged. MicroRNAs are implicated in the control of many fundamental cellular processes; if aberrantly expressed, they are associated with the clinical-pathological features of many diseases. They are able to regulate gene expression on faraway target cells reaching different district of the body and interacting with different pathways [3]. Assuming that AD can be related to microRNA dysfunction, the possibility of some microRNAs alterations in the plasma or serum of AD patients has not yet been explored [4].

Many environmental, occupational or life style risk factors, like exposure to different electromagnetic fields (EMFs), have been hypothesized to increase the risk for AD [5]. Considering the ubiquity of EMFs in our everyday environment, such association would be of high relevance for public health. EMFs have been suggested to increase the risk of several human diseases, although the conclusions are not univocal and certain [6, 7]. Recently, the correlation between EMFs exposure and its negative effects on the nervous system has been supported by occupational studies, which have focused the attention on AD [8]. On the other hand, an intriguing study reports that long-term exposure to microwave frequency EMFs produces cognitive-protective and cognitive-enhancing effects in a murine model of AD [9]. An electromagnetic wave could affect the redox status of the cells, thus evoking general stress responses [10]. The underlying molecular mechanisms is still largely unknown. Research on animals showed positive effects with disappearance of amyloid aggregations; patients exposed to trans-cranial EMF stimulation at different intensity, has shown significant cognitive improvements, as well as improvements in movements.

Our aim is to integrate and analyze clinical, biomolecular and environmental data related to AD patients in order to identify and characterize AD- associated mechanisms involving microRNA sequences, and to determine if their might be beneficially modulated by EMF, or EMF may represent an additional risk factor.

II. COLLABORATION

These investigation will be based on multidisciplinary collaboration strategies, including molecular and cellular biology, physiology, medicine, bio-informatics and electronic engineering. With this interaction, we propose to reach the following tasks by developing a highly integrated and multidisciplinary study. The possibility given by this collaboration is to organize different kind of evaluations and experiments, in order to apply the most recent techniques and acquisitions in different fields of investigations. We found that this kind of approach can be extremely useful while pursuing such complex goals.

III. SCIENTIFIC OBJECTIVES AND APPROACH

The aim is to investigate AD through a in-silico representation of pato-biological mechanisms linked to the disease onset and of the effects of EMF on disease progression, following the Virtual Physiological Human concept, aimed at a systemic approach to disease management. The following research activities will be carried out to identify of the AD-related biomarkers in specific cellular lines (AD model) and evaluate microRNA dysfunctions in biological samples from AD patients. The next step is identification of the impacts of EMF on microRNA modulations and validation of the EMF effects on disease evolution, through the application of EMF to specific patients’ cohorts. We will
focus on both ELF and RF frequency ranges in different operation conditions: continuous, pulsed and modulated waves.

Our approach to disease model definition will follow two main research lines: use of bio-molecular analysis techniques for the provision of high-throughput biomarker and bio-molecular pathways discovery and integration of psycho-physiological measurements, clinical evaluations and bio-molecular data with environmental information. The model will be built in two steps: an in vitro analysis of the genomic aspects of AD cells and a following ex vivo analysis to verify on patients the validity of the model. The effects of EMF exposure on disease evolution will be examined through the evaluation of genomic markers and the measurement of psycho-physical parameters. The ex-vivo research will be performed on comparable patients’ cohorts (exposed vs control), to identify not only an interaction mechanism but also a predictive model of disease evolution of the different subgroups. To this end new suitable electromagnetic exposure systems, if not already available, will be studied and applied.

The results may be used in clinical practice for personalized therapy.

IV. METHODOLOGICAL STRATEGY

PHASE 1
- In vitro exposure to EMFs of cells derived from AD experimental models;
- Cellular and molecular characterization of EMFs effects;
- Identification of miRNAs changes associated with EMFs in AD experimental models.

PHASE 2
- Characterization of beneficial electromagnetic exposure for the treatment of Alzheimer’s disease (AD), in terms of operating modalities and characteristics of the exposure system previously chosen according to the phase 1 results;
- Characterization of miRNAs expression modulated by EMFs associated to better AD neurological responses.

V. PRELIMINARY DATA

Literature data report that miR-30a negatively regulates Beclin 1 expression: this effect is mediated via the miR-30a-3p consensus sequences contained in the 3'-UTR of the BECN1 gene [11]. Therefore, we investigated Beclin 1 protein expression after EMFs treatment in SH-SY5Y cells and compared to untreated (CTR) cells. Beclin 1 expression significantly increased of about 40% following 6 hours EMFs stimulation, while after 3 hours of exposure no changes were observed. Moreover, EMFs exposures do not affect cell proliferation. It was reported that miRNA-17 negatively regulate APP expression [12]. Interestingly, in other preliminary obtained results, we indicate that 24 hours exposure to either 1μM Aβ1-42 peptide or EMFs (75 Hz) increases the expression of miR-17 in SH-SY5Y cells, and that the concomitant exposure to both EMFs and Aβ1-42 leads to a further up-regulation of this miRNA [13]. Furthermore, miRNA-17 and miRNA-30a expression levels are directly modulated by EMFs in blood cell culture systems from AD patients [14].

VI. DISCUSSION

We provide preliminary results produced by our network of labs supporting the rationale of the project. In particular we present data evidencing that in human SH-SY5Y cells a prolonged EMFs stimulation promotes the non-amyloidogenic processing of APP and that EMFs treatment in SH-SY5Y cells affects microRNA30a expression and one of its target proteins (Beclin 1).

BIBLIOGRAPHY

[13] Comincini and Pascale, personal communication
[14] Ricevuti and Venturini, personal communication