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The involvement of the brain microenvironment in Alzheimer's Disease

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Abstract

The recent decision by some "pharma company" to abandon the research in the field of Alzheimer's Disease should not scare the patients and their familial: many other companies and research institutes are strongly working with the attempt to find a cure, a preventive treatment and efficient early diagnosis tools. On the other hand, the risen white flag should worry the same researchers: it is the sign that, in general, a wrong approach has been probably pursued. The failure of the current clinical trials relies on the attempt to treat the neurodegenerative process targeting single effectors of the Disease. It is becoming more and more evident that, as a multifactorial disease, Alzheimer's has to be considered as a systemic pathology in which different biochemical and molecular pathways are involved as well as different cellular population or even different tissues. The road to approach to Alzheimer's through systems biology or medicine is maybe still long, but we already have the background to consider the role of the whole brain microenvironment in the onset and progression of the disease. Glial cells, brain vasculature and blood-brain-barrier clearly play a relevant role in Alzheimer's Disease through the production of several molecules that can influence the (patho)physiology of the neuronal cells. Particularly promising seems the study of the possible epigenetic modifications induced in neurons by the alterations of the brain microenvironment.

Keywords: Alzheimer's Disease; microenvironment; glial cells; systems biology; epigenetics

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1. Introduction

Some years ago, I was attending a promising plenary lecture at a renowned Alzheimer's Disease (AD) conference. Topic of the lecture was, more or less, "how to prevent Alzheimer's Disease". As a young researcher, attempting at finding a path across the multifactorial nature of the disease, I was eager to be finally illuminated and sceptic that the answer to my doubts, for which no sufficient literature existed, could be in one single speech. Surprisingly, during the lecture I realized that the list of the preventive behaviors looked like "grandma's advice": eat healthy food, don't eat too much, have an active life, keep your brain trained, do physical activity, do not exceed with alcohol, maintain social interactions, stay with friends and so on. At first, that seemed to me a bit *naif*, but in the end, I've realized that these advice accounted exactly for the known risk factors associated to late onset (sporadic) AD. And I realized that the relevance of the lecture, more than ten years ago, was that all the known major risk factors, and related preventive actions, where considered as a whole.

On the anatomical-pathological point of view, AD is characterized by the presence in the brain of two protein aggregates: extracellular deposits of Amyloid-β (Aβ) protein and intracellular aggregates of hyperphosphorylated Tau protein (De Strooper, 2000). Aβ is produced by proteolytic cleavage of the large transmembrane Amyloid Precursor Protein (APP), by two enzymatic activities: β- and γ-secretases. β-secretase, encoded by the BACE1 gene, cuts APP in the extracellular domain.

Then, the γ-secretase (a tetrameric complex in which the peptide encoded by Presenilin1 (PSEN1) represents the active site of the cleavage), cleaves the remaining portion of APP in the intramembrane domain, forming Aβ which is involved in the synaptic activity (Chow, 2010). When overproduced or not efficiently scavenged, Aβ aggregates and forms the extracellular deposits called "senile plaques". Tau is a microtubule-associated protein, responsible for regulating the dynamic equilibrium of the microtubules and, as a consequence, the transport of molecules across the axon and the neuronal function. When hyperphosphorylated, Tau is no more able at binding the microtubules and aggregates to form the intraneuronal "neurofibrillary tangles" (Tepper, 2014). Amyloid plaques and Tau tangles stress the neurons inducing malfunctioning and, eventually, neuronal death with consequent brain atrophy and progressive loss of cognitive functions (Selkoe, 2000).

AD exists in two main forms. The genetic form shows autosomic dominant heredity and is caused by mutations in the APP gene or in genes encoding for the enzymes responsible for APP processing, as BACE1 and PSEN1. Also the presence of the ApoE4 allele, an amyloid transporter, is associated to familial AD at different degrees depending on the hetero- or homozygosity of the allele (Dai, 2017). This genetic form is characterized by early onset (EOAD; 40-50 years of age) and rapid development, but its incidence is limited to 5-10%. The vast majority of AD cases is due to the sporadic form of the disease, characterized by late onset (LOAD; >65 years of age) and slow progression. This for is associated with a very high and heterogeneous number of risk factors including (but not limited to) oxidative stress, inflammation, metabolic disorders, diabetes, cardiovascular and cerebrovascular diseases, brain injuries, behavioral stress, nutrition, environmental factors and lifestyle, with a limited role for genetic variants and polymorphisms (Imtiaz 2014; Nicolia 2015). This complex, non-Mendelian, etiology strongly suggest that epigenetic factors, having the capability to mediate the environmental stimuli, may play a major role in LOAD onset and progression.

2. The failure of the therapeutics strategies

The 2017 report from the Alzheimer's Association has been just released (Alzheimer's Association 2017). According to this report, 5.5 million Americans suffer from AD. By mid-century, the number of people living with AD in the United States is projected to grow to 13.8 million, resulting in nearly 1 million new cases per year. AD result the sixth cause of death in the United States and the fifth cause of death in Americans age ≥65 years.

Despite the huge number of AD patients worldwide, the incredible socio-economic costs associated to patients' caregiving, the enormous efforts made by public and private research institutes, pharmaceutical companies, and the elevated funding available, AD still remains without a cure. Only symptomatic treatments are commonly used, mainly aimed at sustaining the synaptic functions via cholinesterase inhibitors (Anand 2013). Unfortunately, these drugs show significant effect in about only 40% of the treated patients and their efficacy declines with the lasting of the treatment.

The search for a cure of AD became a priority of many pharmaceutical companies during the last 20 years. The vast majority of the experimental therapies have been aiming at inhibiting the initiators of pathological Aβ and tau aggregates as well as critical Aβ secretases and critical kinases in tau hyperphosphorylation (Tam 2018). Thousands of promising drugs and molecules have been tested in preclinical research, hundreds of clinical trials have been started, only few arrived to the Phase III, but no one, so far, succeed (Gold 2017). Not surprisingly, according to the for profit nature of the research carried on by pharmaceutical companies, the unbalance between the huge economical efforts and the repeated failure of the trials recently induced Pfizer and, few months before, Merck to announce the abandon of the Alzheimer's research. This decision, by two of the most known and strong multinational companies, created a vast echo even outside the scientific community, together with surprise, criticisms and great worries in the patients, their familiars and in the public society. On the one hand, if the patients should feel reassured by the consideration that many other companies are still actively working on possible AD therapies, diagnostic tools and that several clinical trials are still running out; on the other, is the scientific community that should not miss the main message underlying Pfizer and Merck decision: we are approaching AD the wrong way.

According to the pathological hallmarks originally described in AD brain, i.e. senile plaques and neurofibrillary tangles, research always focused on these two molecules as the main responsible for onset and progression of the pathology. At the beginning, the two approaches were even separated and two different theories were promoted, each one in contrast with the other.

The "amyloid hypothesis" (Selkoe, 2000), and the "tau hypothesis" (Braak, 1986) had her own disciples organized, respectively, in "βaptists" and "Tauists" (Trojanowski, 2002). After a while, the two factions eventually took advantage of the evolving knowledge on AD and realized that the two pathways (Aβ processing and accumulation, and Tau hyperphosphorylation and accumulation) were reciprocally connected and interdependent (Muder, 2002). Moreover, many other different molecular pathways and physiological processes have been found to be implicated in AD pathogenesis: oxidative stress (Chen, 2014), neuroinflammation (Latta, 2015), traumatic brain injury (Mendez, 2015), metabolic syndrome and diabetes (Businaro, 2012), cardiovascular diseases (de Bruin, 2014), nutrients and lifestyle (Barnard, 2014), environmental stresses (Nicolia, 2015), epigenetic factors (Mastroeni 2011; Fuso 2011).

3. Looking at the brain microenvironment

Despite that, strategies leading the studies on AD therapies remained so far stuck on the search for individual druggable targets: mainly Aβ and its proteolytic enzymes β- and γ-secretases (BACE1 and PSEN1, respectively), or Tau phosphorylase GSK3β (Schneider, 2014). The consequence is that, unfortunately but not surprisingly, all the molecules or intervention tested so far, after incredibly promising results in the preclinical trials, failed to succeed. Rather than discourage the future researches, however, these failures should help us to learn the lesson: it is not a winning strategy to treat a multifactorial and complex disease by targeting a single molecule or a single pathway associated to it (Toyn, 2014; De Strooper, 2015; Soejitno, 2015; Gold, 2017). Furthermore, despite the AD pathology mainly affects neurons, it is reasonably to considered that also other cell types contribute to (or, at least, are associated with) the disease. This concept stresses out the importance of looking at the 'whole' brain, with its different cellular components, when hypothesizing and verifying possible treatments. Recent evidences, in particular, point out the role of the microglial microenvironment and AD-associated neuroinflammation. The neuroinflammatory response in course of AD is a well-known event associated to the neurodegenerative process (Latta, 2015), which is induced by the accumulation of Aβ overproduction and aggregation. On the other hand, the possible causative role of neuroinflammation in AD onset, although postulated, remained not clearly ascertained. Neuroinflammatory response is managed by microglial cells and is normally activated, under physiologic conditions, to digest infectious cells or molecules and damaged cells and myelin. Microglia are the resident immune cells of the brain and they play multiple physiological roles, including maintenance of the brain's microenvironment homeostasis. In the injured brain, activated microglia migrate to the inflamed site, where they remove neurotoxic elements by phagocytosis; in addition, microglia support neurons in their functions of message transmitters. When microglia has to cope with the overproduction of Aβ and the plaque spreading, pro-inflammatory signals are overproduced. The over-activation of microglial cells causes the increased inflammatory response that fails to distinguish between healthy and diseased structure, causing excessive degradation of myelin, inducing the degradation of the synaptic structures, worsening and accelerating the neurodegenerative process (Abbott, 2018; Heneka, 2014). To add insult to injury, it was recently demonstrated that over-activated microglia is capable of releasing factors that drive the seeding of new senile plaques. As a matter of facts, activated microglia releases waste produces by inflammasomes in tiny aggregates called specks. Specks have the potential to initiate the seeding of new Aβ oligomers, causing the spreading of the disease across the brain, in a sort of vicious circle in which toxic Aβ induces microglia activation and neuroinflammation that, in turn, promotes further Aβ production, seeding and spreading (Venegas, 2017). This finding was based on the previously demonstrated role of NLRP3 inflammasome in Aβ pathology progression. After activation, NLRP3 recruits the adaptor protein ASC (Apoptosis-associated Speck-like protein containing a CARD) triggering its fibrillar assembly. ASC fibrils, in turn, recruit caspase-1 leading to autoproteolytic activation and subsequent assembly of ASC fibrils into a large paranuclear ASC speck that can promote Aβ aggregation (Lu, 2014). When ASC specks were injected in the hippocampus of APP/PSEN1 transgenic mice, the spreading of Aβ pathology across the brain of the mice was observed. On the contrary, the spreading was not observed after injection of brain homogenates obtained from APP/PSEN1 mice in the hippocampus of ASC-deficient APP/PSEN1 mice (Venegas 2017). Therefore, these evidences clearly support the concept that the activation of the inflammasome is causative of senile plaques seeding and spreading (Fig. 1).

Besides the fundamental advance that foster the knowledge of the role of neuroinflammation in neurodegenerative diseases, these results also add, on a more

general point of view, a relevant brick to the concept that the microenvironment is a fundamental player in neurodegeneration. Increasing evidence sustains the idea that cellular microenvironment creates the conditions that induce the development of the disease. The idea that the microenvironment is fundamental in the developing (and even in the onset) of the disease, is a concept already established in the tumor progression (Bizzarri, 2014). Interestingly, studies on cancer-associated inflammation demonstrated that myeloid-derived suppressor cells (MDSCs) influence the immune escape of cancer cells, and that immunosuppression is not limited to tumors (Salminen, 2018).

AD brain, for example, express a wide range of inflammatory chemokines and cytokines, which could recruit and expand MDSCs and thus generate an immunosuppressive microenvironment in inflamed AD brains (Salminen, 2018). It is ascertained, for example, that activated microglia may play a potentially detrimental role by eliciting the expression of pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) influencing the surrounding brain tissue (Wang, 2015; Lopez-Gonzales, 2015). Therefore, activation of microglia results in a severe alteration of brain microenvironment not only due to the plethora of secreted cytokines, chemokines

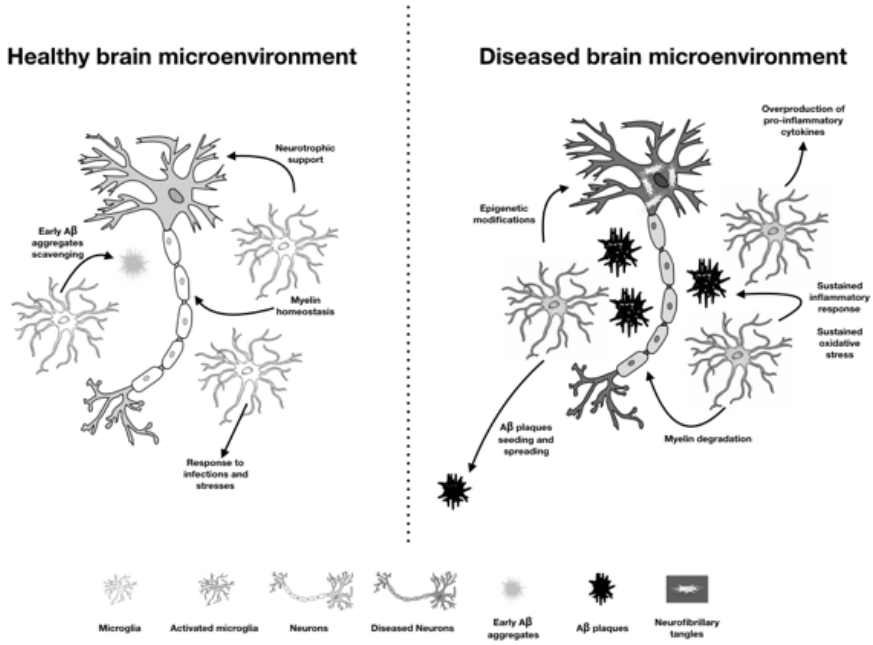

Fig. 1. Schematic representation of the role of microglia, as part of the brain microenvironment, in healthy and diseased brain conditions typical of Alzheimer's Disease. Brain microenvironment, as a consequence of microglia activation, causes sustained oxidative stress response and sustained neuroinflammation, with consequent worsening of the presence of protein aggregates (senile plaques and neurofibrillary tangles) in the brain of AD patients. Moreover, microglia can induce further modulation of gene expression via epigenetic mechanisms and can cause the seeding and spreading of amyloid plaques. New therapeutic approaches should take into account the cross-talk between neurons and brain microenvironment, by designing multi-target drugs able at modulating the different molecular pathways involved in Alzheimer's Disease.

or ROS, but also as a consequence of the increased turnover of neuroprotective endogenous molecules, such as retinoic acid (RA), underlying AD pathogenesis and preceding or facilitating the onset of AD (Regen, 2017).

The down-regulation of energy metabolism is a further example demonstrating the importance of microenvironment in AD. For a while, metabolic changes have been considered merely as a consequence of mitochondrial damage due to oxidative stress, but the non-existence of enhanced response to oxidative stress and the down-regulation of patterns of the electron-transport chain at different AD stages suggest that the down-regulation of energy metabolism in AD is a protective response of neurons enacted by the reduced level of nutrient and oxygen supply within the microenvironment (Sun, 2012). The high apoptotic events occurring at the late stages of AD are presumably driven by the conflict between the lowered energy metabolism and the increased regulatory/ repair mechanisms.

4. The role of epigenetics

These data stress the link existing between the multifactoriality of AD the role of brain microenvironment in the onset and progression of the pathology. Both these two concepts, i.e. multifactoriality and environmental influence, call in cause the epigenetic modifications as possible mediator of the disease (Nicolia, 2015). DNA methylation, in particular, showed the potential of orchestrating the interconnections between different molecular pathways associated to the disease (Fuso, 2011). Although epigenetic mechanisms are still largely associated to differentiating tissues and replicating cells, also the terminally differentiated cell is subjected to "environmental" stimuli (originated either from the organism itself or from the external environment) able to induce changes in gene expression through epigenetic mechanisms. In our laboratory, we produced evidence that DNA methylation regulates (or at least is associated to) Aβ overproduction and aggregation (Fuso, 2012), Tau phosphorylation (Nicolia 2010), pro-inflammatory cytokines IL-1β and IL-6 production (Nicolia, 2017; Dinicola, 2017). It is interesting to note that almost all risk factors associated to AD are known to be causative of epigenetic modulation (Fuso, 2018). It is easy to understand that increased lifespan is associated to higher risk of encounter with environmental stimuli during the adult age and, therefore, the risk of undergoing epigenetic modifications — eventually leading to diseased aging — is higher. Fortunately, epigenetic modifications are, by definition, reversible. Consequently, they are also potential targets for pharmacological interventions aimed at re-establishing the correct epigenome. Recent evidence suggest that also neurogenesis, a process strictly correlated to the homeostasis of the brain microenvironment, is regulated by epigenetic mechanisms. Based on neurogenesis, a number of therapeutic strategies have shown the potential to promote ex novo neuronal generation that could cope the neurons loss in AD, thus improving cognitive function through epigenetic modifications. This can represent another interesting target for the therapy of AD by stimulating neurogenesis using epigenetic strategies (Li, 2016).

5. Conclusions

In conclusion, a large body of evidence stress the idea that microenvironment changes are associated to neurodegenerative disorders and emerging data indicates that these changes can be even causative of the pathology or can accelerate and worsen the progression of neurodegeneration. On this basis is it possible to postulate that multi-target neuropharmacologic approaches restoring the brain's microenvironment can result more effective in respect to the single-target approaches applied so far (Nesi, 2017). Multitarget drugs showed increased interest and application over the last decades since they offer the potential of modulating intricate network effects at the same time with the benefits of a single-molecule therapy. New strategies explore bi-topic inhibitors, for example a single drug acting on different sites of the acetylcholinesterase enzyme to produce at least two different activities, or multitarget drugs acting on multiple therapeutic targets (Pérez, 2015). Acetylcholinesterase inhibition and amyloid pathways are two pivotal features in multitarget design strategies (Rosini, 2016). The chronic "innate neuroinflammation" may therefore provide a valuable target for preventive and therapeutic strategies, since it has the potential to restore the homeostasis of microglial cells and brain microenvironment. Epigenetic therapies, already used in other pathologies including cancer, have also the potential to modulate different pathogenic pathways in AD at the same time (Fuso, 2011). More interestingly, epigenetic intervention can also be imagined in the prevention of the pathology through the "correction" of the environmental factors (nutrition, lifestyle, stresses) that can modify the epigenome. Accepting the idea that a multifactorial disease, like AD, has to be analyzed taking into account the brain microenvironment and has to be treated by multitarget drugs, further pushes our horizon and pave the road to consider the systems biology approach in the study of the disease (Rollo, 2016). The reductionist approach, a specific feature of the current scientific mainstream, have greatly contributed to deciphering the basics of the biology of aging so far. At the same time, this approach let us blind versus the fundamental mechanisms for many identified drugs and pathways (McCormick 2017). In the era of "omics", the metabolomic analysis will gain increasing relevance in studying the systemic changes associated to neurodegeneration and in identifying targets and markers for the development of multitarget drugs (Wilkins, 2018). The new holistic systems-level approaches, at both the experimental and computational level, offer the potential to disclose new fundamental basic mechanisms and functional networks, leading to the characterization of mechanism-based molecular signatures. This approach will also allow the characterization of AD subtypes and stages, toward targeted interventions according to the evolving precision medicine paradigm (Castrillo, 2018), as well as already proposed for other diseases (Kzhyshkowska 2017).

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