PERSPECTIVE

Not only a bad guy: potential proneurogenic role of the RAGE/NF-κB axis in Alzheimer's disease brain

The receptor for advanced glycation endproducts (RAGE) is a receptor of the immunoglobulin superfamily of cell surface molecules which plays important contributions under both physiological and pathological conditions. Over the years extensive research work supported the detrimental role of RAGE in Alzheimer's disease (AD) pathophysiology, ranging from its involvement in beta amyloid (Aβ) brain influx and clearance, neurodegeneration, neuroinflammation, and promotion of synaptic dysfunction. Based on such compelling evidence, preclinical and clinical studies have supported the concept that RAGE inhibitors could represent a useful target for AD treatment (Schmidt et al., 2001; Srikanth et al., 2011).

RAGE is a multifunctional receptor which is expressed in different isoforms and has a highly diverse ligand repertoire, including high mobility group box-1 protein (HMGB-1), Aβ, S100B. Evidence of overactivation of the RAGE-mediated signalling pathway in AD has been collected. Indeed full length RAGE expression is enhanced in both neurons and microglia of AD brain. Conversely, expression of soluble RAGE isoforms, which can function as decoy receptors, is significantly reduced in brain and plasma of AD patients compared to controls (Srikanth et al., 2011). RAGE not only mediates Aβ entry in the brain compartment, but its activation by Aβ can participate in neurodegeneration and neuroinflammation associated with AD. Purified RAGE binds, with nanomolar affinities, oligomeric and aggregated, fibrillar and non fibrillary, Aβ forms, but oligomeric Aβ activates RAGE more robustly than monomeric Aβ and this interaction triggers neurodegeneration and neuroinflammation (Schmidt et al., 2001). In addition to that, RAGE overexpression in microglia exacerbates neuroinflammation and amyloid deposition in the hippocampus and cortex of mutant amyloid precursor protein (APP)/RAGE mice (Srikanth et al., 2011).

RAGE-Aβ interaction activates several downstream signaling events, including nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB)-mediated events. Interestingly, the RAGE promoter region contains NF-κB consensus sequences, allowing a positive feedback loop that may further amplify inflammatory and deleterious responses in AD pathology. Indeed *in vivo* neuronal RAGE binds to Aβ and upregulates RAGE expression levels, followed by increased Aβ production, neuronal toxicity and synaptic dysfunction (Arancio et al., 2004). In double transgenic mutant APP/RAGE mice characterized by neuronal overexpression of RAGE, Aβ-induced neuronal dysfunction and impaired spatial learning/memory are greatly enhanced, compared to the single mutant APP mouse line. In parallel, NF-κB nuclear translocation is increased in the cerebral cortex of double transgenic mice compared to their mutant APP counterpart (Arancio et al., 2004).

HMGB-1, another RAGE ligand, is also overexpressed in AD. Among other deleterious effects, HMGB-1 can bind Aβ42 oligomers, stabilize their aggregation and induce dysfunction in microglial Aβ phagocytosis. Increased HMGB-1 expression in AD brain may also contribute directly to cognitive impairment, since intracerebroventricular injection of HMGB-1 in mice results in impairment of non spatial-long-term memory (Mazarati et al., 2011). Interestingly, such HMGB-1-mediated effects require RAGE interaction.

Altogether, the experimental data supporting a detrimental role of RAGE in the pathophysiology of AD led to the idea that interference with its function may represent a novel therapeutic strategy. Small molecules which can cross the blood-brain barrier were indeed designed as RAGE-specific inhibitors and even as positron emission tomography (PET) ligands (Cary et al., 2016). One such compound, FPS-ZM1, when administered to aged transgenic mice carrying the Swedish APP mutation, resulted in inhibition of RAGE-mediated influx of circulating Aβ40/42 into the brain, reduced neuroinflammation and improved cognitive performance (Deane et al., 2012). Similarly, in another transgenic AD animal model, chronic oral treatment with the RAGE inhibitor PF-04494700 resulted in a significant reduction in both inflammatory markers and amyloid burden (Sabbagh et al., 2011). Unfortunately, despite PF-04494700 was safe and well-tolerated in a Phase I study (Sabbagh et al., 2011), in a subsequent clinical trial mild to moderate AD patients treated with 20 mg/d of PF-04494700 showed increased cognitive decline and adverse events at 6 months (Galasko et al., 2014). At present, the reasons for such disappointing results are not clear. One possibility would be the low predictivity of current animal models which may more closely recapitulate familial rather than sporadic AD. Herein we would like to propose, based on findings generated in our laboratory, that an underestimated complexity in the functional role of RAGE in AD pathophysiology may also explain conflicting results of studies with RAGE inhibitors.

A few years ago, our group identified RAGE expression in a subpopulation of undifferentiated neural progenitor cells (NPC) in the adult neurogenic region referred to as subventricular zone (SVZ). Moreover, we demonstrated that several RAGE ligands, including HMGB-1, significantly increased, *via* RAGE activation, proliferation and neuronal differentiation of SVZ neural progenitor cells. Interestingly, the proneurogenic activity of RAGE ligands in NPC was mediated by activation of the NF-κB pathway. These data were in line with additional work performed in our laboratory, suggesting the critical involvement of NF-κB-mediated pathways in the regulation of adult neurogenesis (Bortolotto et al., 2014). Based on these initial findings, we extended our investigation to another adult neurogenic region, the subgranular zone (SGZ) of the dentate gyrus, and confirmed RAGE expression in adult hippocampal NPC *in vivo*. Additionally, by using an *in vitro* model of adult NPC (Meneghini et al., 2014), we demonstrated that HMGB-1, *via* RAGE engagement, significantly promoted the differentiation of hippocampal NPC toward the neuronal lineage (Meneghini et al., 2013). Also in this region, like in the SVZ, NF-κB signaling lied downstream RAGE activation, since inhibitors of NF-κB p50 and p65 nuclear translocation prevented HMGB-1 effect on NPC differentiation. Additionally, HMGB-1 proneurogenic effects were observed in hippocampal NPC derived from wild type (wt) but not from p50 knockout (KO) mice (Meneghini et al., 2013), pointing to a specific role of this NF-κB subunit. Since the established role of the RAGE/NF-κB axis in the pathophysiology of AD, we then extended our studies to TgCRND8 mice, a well-established murine model characterized by an early onset and rapidly progressing AD-like pathology. To our surprise, hippocampal NPC

from 6–8 month-old TgCRND8 mice gave rise to a significantly higher percentage of neurons, compared to wt-derived NPC (Meneghini et al., 2013). Further, in presence of a neutralizing α-RAGE antibody or of SN-50, an inhibitor of NF-κB p50 nuclear translocation, the increased neurogenic potential of TgCRND8-derived NPC was reduced and became similar to that of wt NPC, suggesting again that activation of the RAGE/NF-κB axis was involved. Interestingly, exposure of wt NPC to TgCRND8-NPC conditioned medium also resulted in a higher number of *in vitro* generated neurons, in parallel with increased p65 nuclear translocation. Conversely, inhibition of p65 nuclear translocation attenuated the proneurogenic effect of TgCRND8 NPC-conditioned media on wt adult progenitors (Meneghini et al., 2013). Altogether these data suggested that soluble factor(s) released by TgCRND8-derived NPC may elicit NF-κB-mediated proneurogenic activity. Based on this observation, we decided to treat wt NPC with nanomolar concentrations of Aβ1–42 monomers, oligomers and fibrils. To our surprise, like HMGB-1, also Aβ oligomers, and not monomers and fibrils, increased, in a concentration dependent manner, the percentage of neurons generated from adult hippocampal NPC. Once again the proneurogenic effects induced by Aβ oligomers were RAGE-mediated and required nuclear translocation of NF-κB p50/p65. As shown for HMGB-1, the proneurogenic effects of oligomeric Aβ were abolished in NPC cultures derived from p50 KO mice. These data, for the first time, suggested that the activation of RAGE/NF-κB axis by Aβ oligomers or by HMGB-1 in adult NPC can potentially contribute to a reparative mechanism which may occur also in AD. Furthermore these data challenged the idea that effects elicited by Aβ oligomers and HMGB-1 are invariably deleterious. This concept is in agreement with previous work showing that infusion of picomolar concentrations of Aβ oligomers can indeed enhance long term potentiation in hippocampal slices and significantly improve spatial long-term hippocampal memory (Srikanth et al., 2011). Interestingly, *via* RAGE activation, other ligands may potentially promote neurogenesis. Intracerebroventricular injection of the RAGE ligand S100B significantly enhances the number of newly generated neurons in the hippocampus of rats subjected to traumatic brain injury and, in parallel, improves cognitive performance in S100B-treated animals compared to vehicle-treated animals (Kleindienst et al., 2005). Based on our findings, it is possible that S100B effects may be mediated, at least in part, through RAGE/NF-κB axis activation.

As previously mentioned, several groups have been actively working on blockade of RAGE as a strategy for therapeutic intervention in AD with, at least so far, disappointing results. The novel proneurogenic role of RAGE/NF-κB axis activation adds complexity to that picture. It suggests the possibility that RAGE engagement by HMGB-1 and Aβ oligomers may contribute not only to neurodegeneration and neuroinflammation, but also regulate adult neural stem/progenitor cell function in pathological conditions where this axis is upregulated, including AD. Despite the vast array of data supporting the idea that RAGE could be an attractive target for pharmacological intervention in neurological disorders, including AD, the complexity in RAGE-mediated responses suggests the need to search for agents that may inhibit RAGE detrimental and maladaptive effects without compromising the potentially adaptive and protective ones like neurogenesis.

Valeria Bortolotto, Mariagrazia Grilli*

Laboratory of Neuroplasticity, Department of Pharmaceutical Sciences, University of Piemonte Orientale "Amedeo Avogadro", Novara, Italy

***Correspondence to:** *Mariagrazia Grilli, M.D., mariagrazia.grilli@uniupo.it.*
***Accepted:** 2016-12-10*
***orcid:** 0000-0001-9165-5827 (Mariagrazia Grilli)*
***doi:** 10.4103/1673-5374.197130*
***How to cite this article:** Bortolotto V, Grilli M (2016) Not only a bad guy: potential proneurogenic role of the RAGE/NF-κB axis in Alzheimer's disease brain. Neural Regen Res 11(12):1924-1925.*

References

Arancio O, Zhang HP, Chen X, Lin C, Trinchese F, Puzzo D, Liu S, Hegde A, Yan SF, Stern A, Luddy JS, Lue LF, Walker DG, Roher A, Buttini M, Mucke L, Li W, Schmidt AM, Kindy M, Hyslop PA, et al. (2004) RAGE potentiates Abeta-induced perturbation of neuronal function in transgenic mice. EMBO J 23:4096-4105.

Bortolotto V, Cuccurazzu B, Canonico PL, Grilli M (2014) NF-κB mediated regulation of adult hippocampal neurogenesis: relevance to mood disorders and antidepressant activity. Biomed Res Int 2014:612798.

Cary BP, Brooks AF, Fawaz MV, Drake LR, Desmond TJ, Sherman P, Quesada CA, Scott PJ (2016) Synthesis and evaluation of [(18)F]RAGER: a first generation small-molecule PET radioligand targeting the receptor for advanced glycation endproducts. ACS Chem Neurosci 7:391-398.

Deane R, Singh I, Sagare AP, Bell RD, Ross NT, LaRue B, Love R, Perry S, Paquette N, Deane RJ, Thiyagarajan M, Zarcone T, Fritz G, Friedman AE, Miller BL, Zlokovic BV (2012) A multimodal RAGE-specific inhibitor reduces amyloid β-mediated brain disorder in a mouse model of Alzheimer disease. J Clin Invest 122:1377-1392.

Galasko D, Bell J, Mancuso JY, Kupiec JW, Sabbagh MN, van Dyck C, Thomas RG, Aisen PS; Alzheimer's Disease Cooperative Study (2014) Clinical trial of an inhibitor of RAGE-Aβ interactions in Alzheimer disease. Neurology 82:1536-1542.

Kleindienst A, McGinn MJ, Harvey HB, Colello RJ, Hamm RJ, Bullock MR (2005) Enhanced hippocampal neurogenesis by intraventricular S100B infusion is associated with improved cognitive recovery after traumatic brain injury. J Neurotrauma 22:645-655.

Mazarati A, Maroso M, Iori V, Vezzani A, Carli M (2011) High-mobility group box-1 impairs memory in mice through both toll-like receptor 4 and receptor for advanced glycation end products. Exp Neurol 232:143-148.

Meneghini V, Bortolotto V, Francese MT, Dellarole A, Carraro L, Terzieva S, Grilli M (2013) High-mobility group box-1 protein and β-amyloid oligomers promote neuronal differentiation of adult hippocampal neural progenitors via receptor for advanced glycation end products/nuclear factor-κB axis: relevance for Alzheimer's disease. J Neurosci 33:6047-6059.

Meneghini V, Cuccurazzu B, Bortolotto V, Ramazzotti V, Ubezio F, Tzschentke TM, Canonico PL, Grilli M (2014) The noradrenergic component in tapentadol action counteracts μ-opioid receptor-mediated adverse effects on adult neurogenesis. Mol Pharmacol 85:658-670.

Sabbagh MN, Agro A, Bell J, Aisen PS, Schweizer E, Galasko D (2011) PF-04494700, an oral inhibitor of receptor for advanced glycation end products (RAGE), in Alzheimer disease. Alzheimer Dis Assoc Disord 25:206-212.

Schmidt AM, Yan SD, Yan SF, Stern DM (2001) The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest 108:949-955.

Srikanth V, Maczurek A, Phan T, Steele M, Westcott B, Juskiw D, Münch G (2011) Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. Neurobiol Aging 32:763-777.