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A novel role for the Receptor for Advanced Glycation End-products in neural progenitor cells derived from adult SubVentricular Zone

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ABSTRACT

The Receptor for Advanced Glycation End-products (RAGE) is a member of the immunoglobulin superfamily of cell surface receptors which interacts with a wide range of ligands, such as High-Mobility Group Box-1 (HMGB-1), S100B, advanced glycation end-products (AGEs). Here we provided evidence for the restricted expression of RAGE in the undifferentiated neural stem/progenitor cells of mouse adult SubVentricular Zone (SVZ) neurogenic region and adult SVZ-derived neurospheres. Additionally, RAGE ligands stimulated both proliferation and neuronal differentiation of SVZ-derived neural progenitor cells (NPC) *in vitro*. NF-κB nuclear translocation occurred upon RAGE activation in SVZ-derived neurospheres and its blockade (by SN-50) or its absence (in $p50^{-/-}$ derived NPC) resulted in the inhibition of the ligand-mediated effects on neuronal differentiation. These novel findings delineate an interesting scenario where the RAGE-NF-κB axis may contribute to regulate adult neural stem/progenitor cell function in physiological and possibly pathological conditions where this axis is upregulated.

Introduction

The Receptor for Advanced Glycation End-products (RAGE) is a multi-ligand receptor of the immunoglobulin superfamily originally described as a cell surface receptor for molecules derived from non-enzymatic glycation and referred to as Advanced Glycation End-products (AGEs) (Neeper et al., 1992). Since then, additional RAGE ligands were identified including amyloid β (Aβ)-peptides and fibrils, constituents of Alzheimer's disease (AD) amyloid plaques, the alarmin HMGB-1 and the calcium-binding proteins S100/calgranulins (Ding and Keller, 2005). Furthermore RAGE was identified as a counter-receptor for the leucocyte β2 integrin Mac-1 (Chavakis et al., 2003). RAGE exists both as membrane-bound and soluble isoforms (sRAGE), with the latter ones acting as decoy receptors that bind circulating ligands (Neeper et al., 1992; Ding and Keller, 2005). Structurally, membrane-bound full length RAGE (FL-RAGE) consists of three extracellular immunoglobulin-like domains (one V- and two C-types), a single transmembrane domain and a short intracellular C-terminal tail, which is essential for intracellular signalling (Huttunen et al., 1999; Dattilo et al., 2007). sRAGE isoforms can be generated by alternative splicing (Hudson et al., 2008; Kalea et al., 2009) or by proteolytic cleavage of FL-RAGE by ADAM10 (Raucci et al., 2008).

In normal adult tissues, RAGE is usually expressed at low levels, except in the lung where it is quite abundant (Hudson et al., 2008; Kalea et al., 2009). Upon ligand accumulation in a tissue, RAGE is commonly upregulated and produces a sustained cell activation through multiple intracellular signalling pathways (Li and Schmidt, 1997; Bierhaus et al., 2001; Schmidt et al., 2001; Ding and Keller, 2005). A vast array of information has been collected on the possible contribution of RAGE activation to several diseases such as diabetes, AD, atherosclerosis, cancer progression and stroke (Schmidt et al., 2001; Arancio et al., 2004; Ding and Keller, 2005; Fang et al., 2010; Takuma et al., 2009). For such reasons, RAGE is viewed as an attractive target for pharmacological intervention in those disorders (Geroldi et al., 2006; Maczurek et al., 2008).

In the adult central nervous system (CNS), RAGE has been shown to be expressed at low levels by neurons, glia, endothelial cells and it has been involved in multiple events including proliferation, neurite outgrowth, migration and apoptosis (Yan et al., 1996, 1997; Huttunen et al., 2000; Lue et al., 2001; Chou et al., 2004; Schmidt et al., 2007; Qin et al., 2008). Among the signalling pathways which lie downstream RAGE engagement are those involving the NF-κB family of transcription factors, whose role in the CNS is widely recognized (Grilli and Memo, 1999). Additionally, NF-κB p50/p65 dimer has been shown to activate an autoregulatory positive feedback loop whereby RAGE itself is upregulated (Li and Schmidt, 1997; Bierhaus et al., 2001). Recently our group provided evidence for the selective expression of NF-κB transcription factors in neurogenic areas of adult mouse brain (Denis-Donini et al., 2005). Moreover, we demonstrated a selective defect in adult neurogenesis in NF-κB p50 knock-out mice (Denis-Donini et al., 2008). Little is known on the possible involvement of RAGE in adult neurogenesis but, interestingly, Manev et al. (2003) reported that the

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receptor is expressed by BrdU-labelled cells in the hippocampal SubGranular Zone (SGZ) after chronic fluoxetine administration, a treatment known to promote neurogenesis (Santarelli et al., 2003). Based on these observations, we decided to investigate the potential role of the RAGE-NF-κB axis in the modulation of adult neurogenesis. We confirmed that *in vivo* RAGE is expressed by the neural stem/progenitor cells in the neurogenic SVZ region of the adult mouse brain. We also characterized *in vitro* the expression of the membrane-bound FL-RAGE isoform in adult mouse neural progenitor cells (NPC). Moreover, we investigated the functional role of the RAGE-NF-κB axis in the proliferation and differentiation of adult NPC.

Results

RAGE is expressed in vivo in the SVZ of adult mice

Immunohistochemical analysis using an N-terminal anti-RAGE antibody confirmed the presence of the receptor in the adult mouse SVZ region (Fig. 1A–I). To further characterize the RAGE-positive cell population we performed double-labelling experiments with GFAP (Glial Fibrillary Acidic Protein), a marker of adult neural stem cells (or type B cells) and of mature astrocytes, and Sox-2 (anti-SRY-related HMG-box gene 2), a transcription factor expressed in the nucleus of undifferentiated stem/progenitor cells (Graham et al., 2003; Brazel et al., 2005). As shown in Fig. 1, a subpopulation of GFAP-positive cells coexpressed RAGE (Fig. 1A–C), while the majority of Sox-2-positive cells were also immunoreactive for RAGE (Fig. 1D–F). Doublecortin (DCX) is a microtubule-associated protein expressed by immature neuroblasts in adult neurogenic areas (Lois and Alvarez-Buylla, 1994). When RAGE/DCX colabelling was performed, no colocalization was observed in the adult SVZ (Fig. 1G–I). To further characterize the nature of the subpopulation of $GFAP^+/RAGE^+$ cells in the adult SVZ, we then decided to performed a triple-labelling experiment with antibodies against RAGE, GFAP and Sox-2. As shown in Fig. 2A, virtually all $RAGE^+/GFAP^+$ cells were also marked by Sox-2 expression suggesting that they may be identified as undifferentiated neural stem/progenitor cells (Brazel et al., 2005).

In order to further analyze the expression of RAGE in $BrdU^+$ proliferating cells, a group of adult CD1 mice were administered a single injection of bromodeoxyuridine (BrdU, 150 mg/kg, intraperitoneally) and sacrificed 24 h later. As shown in Fig. 2B, a triple-labelling experiment with antibodies against RAGE, GFAP and BrdU demonstrated that BrdU immunoreactivity was absent in the $RAGE^+/GFAP^+$ cells, possibly suggesting that this subpopulation could be identified as quiescent neural stem cells (type B cells). Conversely, triple-labelling experiment with antibodies against RAGE, Sox-2 and BrdU (Fig. 2C) showed that several $RAGE^+/Sox\text{-}2^+$ cells were marked by BrdU, possibly suggesting that RAGE is expressed in a

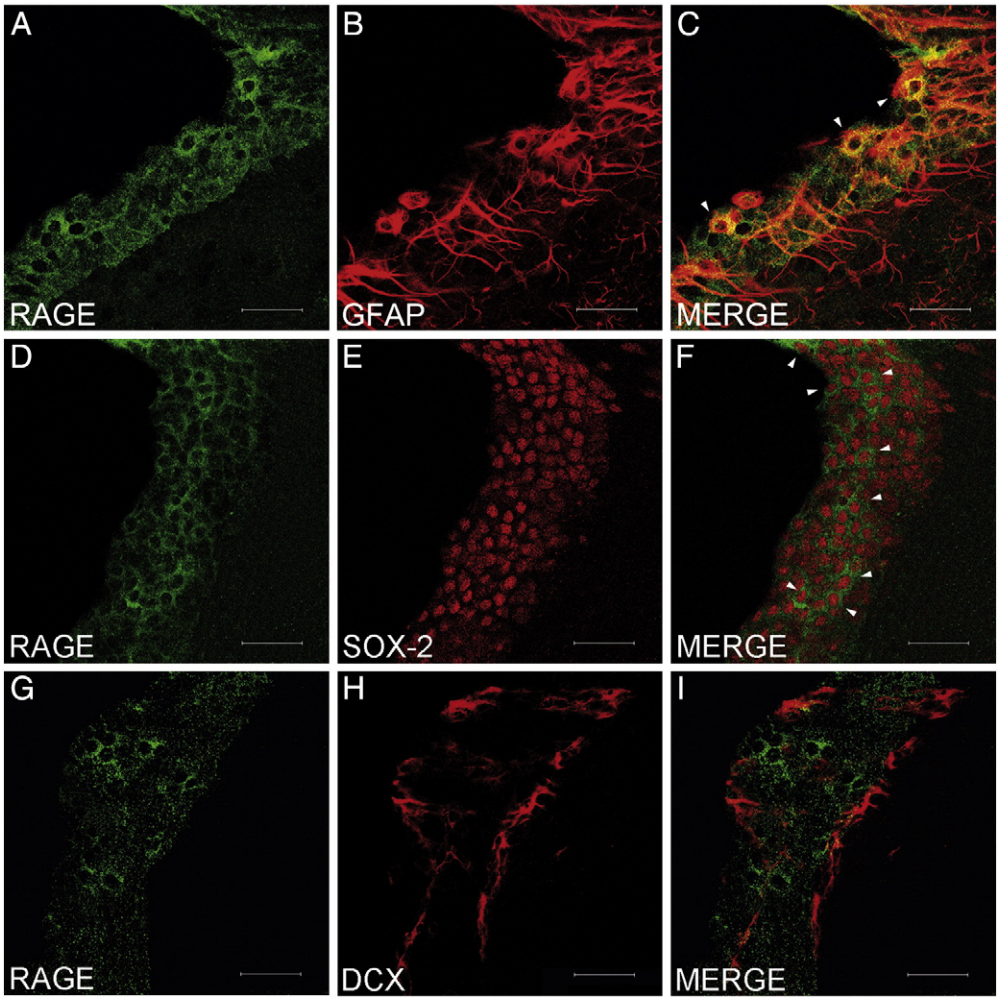

Fig. 1. RAGE is expressed *in vivo* in the neural stem/progenitor cells of the adult SVZ region. (A–I) Representative confocal microscopy images of the adult mouse SVZ region immunostained with antibodies against RAGE (A, D and G, green), GFAP (B, red), a marker of type B cells or mature astrocytes, Sox-2 (E, red), a marker of stem/progenitor cells, or DCX (H, red), a marker of immature neuroblasts. Overlay of the two channels demonstrated that a subpopulation of $GFAP^+$ cells (indicated by arrowheads) coexpressed RAGE (C), the majority of $Sox\text{-}2^+$ cells (indicated by arrowheads) were also immunoreactive for RAGE (F), while no colocalization was observed between RAGE and DCX (I). Magnification = ×800. Scale bars = 37.5 μm.