BRAIN RESEARCH 1620 (2015) 17–28

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Brain Research

Research Report

Extracellular α-crystallin protects astrocytes from cell death through activation of MAPK, PI3K/Akt signaling pathway and blockade of ROS release from mitochondria

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ARTICLE INFO

Article history:
Accepted 9 May 2015
Available online 18 May 2015

Keywords:
α-Crystallin
Astrocytes
Apoptosis
Neuroprotection
Reactive oxygen species
Small heat shock proteins HSPB4 and HSPB5

ABSTRACT

α-Crystallin with two isoforms, αA-crystallin (HSPB4) and αB-crystallin (HSPB5), is found in eye lens, spleen, lung, kidney, cornea, skin, but also in brain. Several studies revealed roles of αA/αB-crystallin in regulating cell viability and protection in the central nervous system. We previously demonstrated that α-crystallin serves as an intracellular protectant in astrocytes. Compared to well-studied intracellular functions of α-crystallin, there is limited proof for the role of α-crystallin as extracellular protectant. In order to clarify protective effects of extracellular αA/αB-crystallin, we exposed astrocytes to the toxic agents, staurosporine or C2-ceramide, or serum-starvation in the presence of αA/αB-crystallin. Extracellular αA/αB-crystallin protected astrocytes from staurosporine- and C2-ceramide-induced cell death. In addition, extracellular αB-crystallin/HSPB5 effectively promoted astrocytes viability through phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) and extracellular signal-regulated kinase 1/2 (ERK1/2), p38 mitogen-activated protein kinases (p38) and c-Jun N-terminal kinases (JNK) signaling pathways under serum-deprivation. Furthermore, αB-crystallin/HSPB5 decreases the staurosporine-mediated cleavage of caspase 3 through PI3K/Akt signaling preventing

Abbreviations: Akt, protein kinase B; EAE, experimental allergic encephalomyelitis; ERK, extracellular signal-regulated kinase; FCS, fetal calf serum; JNK, c-Jun N-terminal kinases; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinases; MEK, mitogen-activated and extracellular signal-regulated kinase; mTOR, mammalian target of rapamycin; P38, p38 mitogen-activated protein kinases; PI3K, Phosphatidylinositol 3 kinase; RBM, rat brain mitochondria; ROS, reactive oxygen species; sHSPs, small heat shock proteins; STS, staurosporine; UVA, ultraviolet A; WST, water soluble tetrazolium

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http://dx.doi.org/10.1016/j.brainres.2015.05.011
0006-8993/

apoptosis of astrocytes. Thus, the current study indicates that extracellular αA/αB-crystallin protects astrocytes exposed to various harmful stimuli. Furthermore, application of αB-crystallin/HSPB5 to isolated rat brain mitochondria inhibits ROS generation induced by complex III inhibition with Antimycin A.

1. Introduction

α-Crystallin exists as large aggregates, composed of two types of the related subunits αA- and αB-crystallin that are highly similar to the small (15–30 kDa) heat shock proteins (sHSPs) (de Jong et al., 1988; Ingolia and Craig, 1982). α-Crystallin was first found as major structural protein of the mammalian lens (de Jong et al., 1989). Accumulating studies have confirmed the protein expression of α-crystallin in brain, spleen, lung, kidney, cornea, and skin, and a role of α-crystallin in regulation of cell survival, and functions in the central nervous system (Bajramovic et al., 1997; Iwaki et al., 1990; Maddala and Rao, 2005; Srinivasan et al., 1992). sHSPs αA/HSPB4 and αB-crystallin/HSPB5 have been demonstrated to exert multiple functional effects on retinal and other neurodegenerative diseases (Fort and Lampi, 2011 and references therein). This article outlines that crystallins are involved in numerous pathologies, particularly those showing major inflammatory responses and it summarizes that crystallins inhibit cell apoptosis. The widespread implications of α-crystallin in retinal functions and diseases have been comprehensively reviewed (Kannan et al., 2012).

As molecular chaperone, α-crystallin plays functional roles in the extracellular matrix and the plasma membrane, but also in intracellular organelles including the nucleus. For example, α-crystallin targets extracellular components decreasing the damage to cells (Sharma et al., 1987). Extracellular application of α-crystallin promotes survival and proliferation of rat olfactory ensheathing cells (Wang et al., 2012a). Apart from this, α-crystallin can protect the plasma membrane from rupturing induced by various stimuli (Cobb and Petrash, 2000). In the intracellular environment, α-crystallin stabilizes proteins under stress and prevents their denaturation (Nicholl and Quinlan, 1994). Later it was confirmed that α-crystallin binds to denatured proteins to promote the recovery of protein activity (Andley et al., 2000). Other studies provide evidence that α-crystallin translocates from cytosol to the nucleus and regulates gene expression (den Engelsman et al., 2004, 2005). The signaling pathways integrated in the physiological effects mediated by α-crystallin *in vitro* and *in vivo* have been well documented. For instance, αB-crystallin/HSPB5 prevents apoptosis in lens epithelial cells caused by ultraviolet A (UVA) through repression of UVA-induced activation of the Raf/mitogen-activated and extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway, whereas αA-crystallin/HSPB4 activates the Akt survival pathway to block the UVA-induced apoptosis. Beside this, it was found that the calcium-activated Raf/MEK/ERK signaling pathway mediating p53-dependent apoptosis is suppressed by αB-crystallin/HSPB5-activated Ras (Li et al., 2005). *In vivo* investigations showed that injection of α-crystallin into the rat vitreous inhibited RhoA protein activity and the phosphorylation of both cofilin and myosin light chain, and therefore promoted the axonal growth (Wang et al., 2012b). Furthermore, α-crystallin has been shown to bind to the proapoptotic molecules p53, Bax and Bcl-X(s) to inhibit the translocation of these proapoptotic molecules from cytoplasm to mitochondria, and thus α-crystallin blocks the release of cytochrome c from mitochondria to activate apoptosis (Kamradt et al., 2001; Liu et al., 2007; Mao et al., 2004; Wang et al., 2012b).

Reactive Oxygen species (ROS) are well known by-products of the normal metabolism. Mitochondria are believed to be a key target of oxidative damage, but represent also the major producer of ROS in cells. The largest part of mitochondrial ROS is generated at the electron transport chain (Chance et al., 1979). Mitochondrial ROS can be produced under different stimuli, such as non-esterified polyunsaturated fatty acids (Schönfeld et al., 2011). Increased mitochondrial ROS production damages the brain, which contributes to neuronal damage in Alzheimer's disease (Sullivan and Brown, 2005), Parkinson's disease (Cassarino et al., 1997), and stroke (Warner et al., 2004). The potential mechanisms accounting for these pathologies are decreased ATP production, abnormal mitochondrial membrane potential, permeability transition pore activation, and reduced Ca^{2+} capacity (Kirkinezos and Moraes, 2001).

αB-Crystallin/HSPB5 colocalizes and interacts with mitochondria (Yaung et al., 2007) and protects retinal pigment epithelium cells against endoplasmic reticulum stress by restoring mitochondrial functions (Dou et al., 2012). The astrocytes from mice suffering from inflammation showed a decreased level of ROS, when the animals were pre-treated with α-crystallin, emphasizing that α-crystallin may serve as a potent pharmacological reagent in neuroinflammation (Masilamoni et al., 2005a). During the early phase of experimental autoimmune uveitis, upregulation of α-crystallin was found to enhance resistance against mitochondrial oxidative stress and stress-mediated apoptosis (Rao et al., 2008).

We previously demonstrated that cellular overexpression of α-crystallin and the phosphorylation of α-crystallin protect astrocytes from cell death induced by the agents C2-ceramide and staurosporine (Li et al., 2009; Li and Reiser, 2011). Compared to the large number of reports describing the functions of α-crystallin in the intracellular environment, there is still limited information for understanding the functional role of α-crystallin, when it is applied as extracellular protein. Several studies reveal that sHSPs which are induced by endogenous or exogenous factors and exhibit extracellular functions can be released from cells. For instance, the pathways of exosomal secretion of αB-crystallin/HSPB5 from primary human retinal pigment epithelium cells and the ARPE-19 cell line were described (Gangalum et al., 2011; Sreekumar et al., 2010).