Peptidylarginine deiminase and neurodegenerative diseases

Abbreviations

AD: Alzheimer’s disease

CNS: Central nervous system

GFAP: Glial fibrillary acidic protein

NFT: Neurofibrillary tangles

MAP2: Microtubule-associated protein 2

MBP: Myelin basic protein

Nef3: Neurofilament 3

PAD: Peptidylarginine deiminase

RT-PCR: Reverse transcriptase-polymerase chain reaction

SP: Senile plaque

Introduction

Neurodegenerative diseases, such as Alzheimer’s disease and Multiple Sclerosis, are a group of progressive conditions, which affect a person’s learning abilities and the day-to-day routine management. Citrullination/deiminasion is a process describing the enzymatic involvement in conversion of arginine residues into citrulline in target proteins. Here, is a review, entailing the main features of the peptidylarginine deiminase (PAD) enzyme family, with a view on its history, presence and future in neurodegenerative disorders.

Background

The amino acid Citrulline was firstly mentioned in 1955 (Smith and Young), and the citrullination process of L-arginine conversion to L-citrulline was firstly described in 1958 (Rogers and Simmonds). Citrullination is a posttranslational process, and also results in a 1Da decrease in size, due to an hydrolytic reaction (Vossenaar et al., 2003; Gyorgy et al., 2006; van Venrooji and Pruijn, 2003). However, it was only in 1977 when peptidylarginine deiminases, the enzymes that are responsible to the citrulline modification, were firstly described (Rogers et al.).

Alzheimer's disease:

GFAP, an astrocyte-specific marker protein, is involved in the progression of Alzheimer's disease via PAD2 citrullination. The increase of PAD2 can be seen in the hippocampi of AD patients. GFAP and vimentin citrullination was also identified by using a 2D gel electrophoresis and MALDI-TOF mass spectrometry. Activation of PAD occurs upon a mass influx of $Ca^{2}+$, and therefore an increase in citrullinated proteins (Keller et al., 2000; Maccioni et al., 2001). The PAD family consists of five known members (1-4 & 6), each is present in a different area in the human body. PAD2 and PAD4 area of activity is the central nervous system (CNS), although PAD2 in larger amounts than PAD4 (Kubilus and Baden, 1983; Watanabe et al., 1988; Terakawa et al., 1991). Yet, the role of the different PADs is still largely understood. PAD3 was found in the root sheaths of hair follicles, as part of the citrullination process of keratinisation (Kanno et al., 2000). PAD4 was found in human myeloid leukemia HL-60 cells, and later in peripheral blood granulocytes (Nakashima et al., 1999; Asaga et al., 2001). PAD2 and PAD4

are also present in the myelin sheath. In fact, hyper-citrullination of myelin basic protein (MBP) is now known to result in myelin sheath loss in multiple sclerosis patients (Moscarello et al., 1994; Wood et al., 2008; Musse et al., 2008). Moreover, PAD4 is also involved in histone deimination in brain tissues of such patients (Mastronardi et al., 2006). Following immunocytochemical studies, PAD2 have been detected in glial cells and astrocytes (Asaga and Ishigami, 2000; Vincent et al., 1992; Asaga and Ishigami, 2001), microglial cells (Vincent et al., 1992; Asaga et al, 2002), oligodendrocytes (Akiyama et al., 1999), and Schwann cells (Keilhoff et al., 2008). Furthermore, during hypoxic insult (Asaga and Ishigami, 2000) and during kainic acid administration (Asaga and Ishigami, 2001; Asaga et al., 2002), neurodegenerative regions shown to have PAD2 activity and deimination of various proteins. These findings suggest that PAD2 plays an important role in protein citrullination in neurodegeneration. Senile plaques (SPs) and neurofibrillary tangles (NFTs) are the two main forms of protein aggregation, and therefore responsible for neuronal death in AD (Katzman, 1986; Smith, 1998). They are mainly found in the hippocampus and cerebral cortex, when levels of PAD2 were detected to be more than threefold higher in the hippocampus than in the cortex of rat brains (Asaga and Ishigami, 2000). A study by Ishigami et al. (2005) was set to evaluate the involvement of protein citrullination in AD patients' brains, and identified two citrullinated proteins- GFAP and vimentin, by using a two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry. It went on to identify also a citrullinated MBP, again in the hippocampus region of the AD patients.

Multiple sclerosis:

Higher rates of citrullinated MBP were found in the CNS of MS patients than in the CNS of healthy adults (Mastronardi et al., 2006; Nicholas and Whitaker, 2002; Raijmakers et al., 2005). However, similar levels of citrullination have been observed in children under the age of 4. An indication that citrullination is involved in the development of mature myelin (Moscarello et al., 1994; Wood et al., 1996). Due to known increased citrullination, PAD2 and PAD4 rates in myelin from normal-appearing white matter (NAWM) of seven MS patients were examined, and were found to be significantly higher compared to NAWM from six control individuals, using immunoblots with PAD2 and PAD4-specific antibodies (Wood et al., 2008). The anti-citrulline antibody-F95, was also used in the same manner, resulting in the same outcome (Nicholas et al., 2004). The study by Wood et al. (2008) also discovered that PAD2 can citrullinate 18 out of 19 arginine residues, whereas PAD4 can only citrullinate 15 out of 19 arginine residues localised in MBP. It has been found and reported that MPB deimination increases its susceptibility to degradation by proteinases, which are elevated around active plaques and in the cerebrospinal fluid (CSF) (Cuzner and Davison, 1973; Einstein et al., 1972; Richards and Cuzner, 1978). It has been also reported that macrophages and reactive astrocytes are able to produce the proteinase cathepsin D (Allen and McKeown, 1979; Prineas and Wright, 1978). Cathepsin D then produces peptides that contain the immuno-dominant epitopes of MBP, located in the CSF of MS patients (Whitaker, 1977; Whitaker and Granum, 1980). It was then discovered that different MBP strains, containing greater amounts of citrulline per mole of MBP than their counterparts were digested at a much faster rate by cathepsin D (Pritzker et al., 2000). An explanation for this

discovery by Pritzker et al. can be explained by the three-dimensional atomic structure of the human MBP they created. The structure showed a clear correlation between the open conformation of the atomic structure and the higher rate of citrullination in-site, which allows cathepsin D a better access to Phe-Phe linkages in MBP, and so an increased digestion of citrullinated MBP.

Current perspectives

In addition to MBP, GFAP also have shown to be citrullinated in MS. In a study by Nicholas et al. (2004), GFAP citrullination rate in both the NAWM and lesions of patients with SPMS was compared with the white matter of control brain tissue, and was found to be in higher amounts. The findings were obtained using GFAP anti-citrulline antibodies (such as P95) and confocal microscopy. On an extended study by Nicholas et al. (2004), using dual staining wit GFAP anti-citrulline antibodies, it was found that lesions taken from nine MS patients were highly citrullinated compared to white matter taken from six healthy control individuals. Western blotting has also confirmed that the majority of the deimination occurred in GFAP. PAD4 is enable to translocate from the cytoplasm to the nucleus, due to its ability to carry a functional nuclear localisation signal (Nakashima et al., 2002). PAD4 modification can also affect nuclear proteins, histones H2A, H3 and H4, and nucleophosmin/B23 (Hagiwara et al. 2002). Deimination of histones causes change in chromatin confirmation, and therefore gene transcription gets turned “ off” (Shimoyama et al., 2010). In a study by Cuthbert et al. PAD4 was found to inhibit transcription of estrogen-responsive genes in MCF-7 cells. Levels of PAD4 in MCF-7 cells were shown to rise in response to

estrogen, followed by deimination of the pS2 promoter by PAD4, which coincides with downregulation of this gene (Cuthbert et al. 2004). Wang et al. showed that incubation of purified substrates H3 and H4 with PAD4 in vitro generated citrullinated H3 and H4, which coincided with a dramatic reduction in H3 Arg 17 and H4 Arg 3 methylation (Wang et al. 2004). This effect was also mimicked when HL-60 granulocytes were incubated with PAD4 in vivo, in the presence of calcium ionophore. PAD4 was found to be elevated in NAWM from 17 patients with MS, compared to 9 control subjects (Mastronardi et al. 2006). This was shown by fractionation of these samples into membrane-containing, non-microsomal, and nuclear fractions, followed by quantitation of the amount of PAD 1-4 antibody binding. The nuclear fraction contained a 3. 5-fold increase in the level of PAD4 in patients with MS compared to controls. Through western blot analysis using anti-PAD4 antibody, this was attributable to increased PAD4 in the MS NAWM. Using an antibody against citrullinated proteins, this increase in PAD4 was found to be accompanied by an increase in citrullinated proteins in brain tissue taken from patients with MS, whereby strong nuclear labeling in NAWM from MS patients was seen compared to controls (Mastronardi et al. 2006). This increase in PAD4 was also accompanied by an increase in nuclear histone H3 citrullination, as shown by immunostaining of MS and control tissue with an antibody against citrullinated protein, which revealed strong nuclear staining of cells in the MS white matter. These findings were confirmed by western blot analysis, which showed a great abundance of citrullinated H3 in MS NAWM, with only traces in white matter from controls. This citrullination of histones greatly affects the chromatin structure and function, as deimination of arginine residues of histones decreases their positive charge, which

compromises its ability to interact with DNA and possibly resulting in apoptosis of affected cells (Moscarello et al. 2007; Wang et al. 2004). It is not known whether excess citrullination is a primary or a secondary event to the inflammatory process in MS or whether the regulation of PAD isoforms may be part of the genetic susceptibility to MS. Single-nucleotide polymorphisms in the PAD4 gene, also associated with the autoimmune disease rheumatoid arthritis, increase mRNA stability, suggesting that this could result in greater PAD4 protein expression and hence increased citrullination of proteins (Suzuki et al. 2003). Increased PAD2 protein expression in human astrocytes in vitro has also been reported in response to increased intracellular calcium levels when cells were subjected to elevated pressure or in response to hypoxia (Bhattacharya et al. 2006a, b; Sambandam et al. 2004).

A number of pathological processes, including excitotoxicity, occur in the CNS of patients with MS, which would lead to raised intracellular calcium ions in neurons and glia (Shideman et al. 2006; Smith 2007). Large numbers of activated macrophages are present in inflammatory demyelinating sites within MS lesions. Since these cells contain PAD enzymes and there is increased cell death due to raised intracellular calcium ions, this would lead to activation of PAD enzymes when released from dying cells (Bhattacharya et al. 2006a). Thus myelin proteins may be citrullinated both intracellularly, during myelin degradation following phagocytosis, as well as extracellularly, following release of PAD enzymes from dying cells. In addition, significant hypomethylation of the PAD2 promoter has also been found to occur in MS NAWM compared to controls, which may lead to increased PAD2 expression

and subsequent increase in citrullination, as hypomethylation leads to increased gene transcription (Mastronardi et al. 2007).

Future perspectives

Conclusion

In the last 60 years since it was firstly mentioned, the research area of PADs have made a tremendous leap and came about with many important discoveries regarding the mechanisms and pathologies concerning PADs. However, it still seems to be largely unknown and have many possible routes of research. Especially when considering the wide localisation of the PADs throughout the human body. Such areas of research may ask after the origins of the calcium influx to the brain tissues, and the timing of that physiological event; how may the rate of calcium influx affect the rate of neurodegeneration; what would prove to be the most effective PAD-inhibitor treatment, etc.

However, looking at the milestones along the research of PADs, it does seem that the right questions are being asked, and that the available/emerging technologies are suitable for this research.

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