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Pharmacological Research 52 (2005) 5–14

Pharmacological research

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Mediation of cell death by poly(ADP-ribose) polymerase-1

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Accepted 1 February 2005

Abstract

Poly(ADP-ribosyl)ation plays an important role in modulating the cellular response to stress. The extent of poly(ADP-ribosyl)ation, chiefly via the activation of the poly(ADP-ribose) polymerase-1 (PARP-1), correlates with the severity of genotoxic stress and this determines the cellular response. Under mild and moderate stress, it plays important roles in DNA processing and it participates in the proinflammatory/cellular defense via transcriptional regulation. However, severe stress following acute neuronal injury causes the overactivation of PARP-1, which results in unregulated poly(ADP-ribose) (PAR) synthesis and widespread neuronal cell death. Previously, this PARP-1-dependent cell death mechanism was manifest solely through necrosis, but apoptotic mechanisms are also evident. Poly(ADP-ribosyl)ation directly induces the nuclear translocation of apoptosis-inducing factor, which results in caspase-independent cell death significant in many neurodegenerative conditions. Further, the hydrolysis of PAR by poly(ADP-ribose) glycohydrolase (PARG) has a protective role, since the accumulation of PAR leads to cell death by apoptosis. Thus, PAR signaling, regulated by PARP-1 and PARG, mediates cell death. Accordingly, modulation of PAR synthesis or degradation through the targeting of PARP-1 or PARG holds particular promise in the treatment of conditions such as cancer, stroke, and Parkinson's disease.

Keywords: Apoptosis; Poly(ADP-ribose); Excitotoxicity; PARG; Apoptosis-inducing factor

1. Introduction

Cell death mechanisms control many physiologic processes, including nervous system development, autoreactive T cell negative selection, and the elimination of virally infected cells. They also control many pathologic processes, such as cancer, tissue injury, and cell stress. These cell death mechanisms all set into motion many events and extracellular/intracellular signaling cascades, many of which we do not fully understand. Currently, cell death is typically described as being apoptotic or necrotic based upon cellular morphological and intracellular ultra-structural changes. Apoptosis is an energy-dependent form of programmed cell death that has many proteins that either prevent or enable its progression. Structurally, it is characterized by nuclear chromatin condensation, membrane blebbing, and cell fragmentation into apoptotic bodies. Necrosis, on the other hand, is an acute form of cell death usually initiated following energy loss. It is characterized by nuclear disintegration, loss of membrane integrity, appearance of pyknotic nuclei, and cell lysis that is typically followed by an inflammatory response. However, the ability to have a clear differentiation between apoptosis and necrosis can be complicated, since overlap in the criteria can occur. For example, following stroke, trauma, or other neurodegenerative diseases, the morphological characteristics of dying neurons meet the criteria for necrosis, such as pyknotic nuclei, and they can also meet those for apoptosis, such as phosphatidylserine exposure and nuclear chromatin condensation. Therefore, it appears that neurons are capable of specialized death programs following exposure to cytotoxic agents, hypoxia, or ischemia. A better understanding of these events and their regulation should allow the development of successful therapies to induce cell survival which can thus treat or prevent neurodegenerative diseases which cause widespread cell death in the nervous system. Poly(ADP-

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doi:10.1016/j.phrs.2005.02.011

ribosyl)ation, mainly initiated through PARP-1, is a key mediator of cell death in post-mitotic cells. Therefore, this review will focus on the ability of poly(ADP-ribosyl)ation to mediate cell death in the nervous system based upon recent findings. The relevance of this review is to identify therapeutic targets in the treatment of various debilitating and devastating neurological disorders. Many of these conditions have no known treatment regimens outside of supportive care. These disorders include stroke, trauma, and Parkinson's disease.

2. Evidence of the role of poly(ADP-ribose) in multiple cell death mechanisms

2.1. Poly(ADP-ribosyl)ation

Poly(ADP-ribosyl)ation is essential for the maintenance of genomic integrity [1]. Polymers of ADP-ribose [poly(ADP-ribose) or PAR] are synthesized by the poly(ADP-ribose) polymerase family of enzymes (PARPs) using nicotinamide adenine dinucleotide (NAD^+) as substrate with the release of nicotinamide [2]. A diverse array of proteins are acceptors of these polymers, which results in their covalent modification and/or the non-covalent binding of vicinal proteins to the negatively charged sites on each ADP-ribose unit [2]. However, these protein modifications and attractions are only transient due to the rapid action of poly(ADP-ribose) glycohydrolase (PARG), which catalyzes the hydrolysis of PAR into free ADP-ribose [2]. The immediate activation of PARP-1 following DNA damage demonstrates an immediate response to cell stress by poly (ADP-ribosyl)ation, while the short duration of PAR polymer half-life suggests a rapid metabolic cycle implemented by PARP and PARG, two closely coordinated proteins.

Poly(ADP-ribosyl)ation is present in all multicellular eukaryotes with few exceptions. More specifically, PAR synthesis and degradation is present in all mitotic and post-mitotic cells in mammalians [3]. In the central nervous system (CNS), PARP and PARG are present throughout the brain and spinal cord. Each cell type within the CNS contains varying amounts of PAR levels as demonstrated by immunoblotting or immunocytochemistry. PARP-1 activation is present in macroglial cells, such as astrocytes, and phagocytosing microglial cells, but not in resting microglia [4]. Neurons contain high levels of PARP-1 activity following trauma, ischemic injury, or oxidative stress, which explains their extreme sensitivity to PARP-1-dependent excitotoxic cell death mediated by the neurotransmitter glutamate [5]. The ability of poly(ADP-ribosyl)ation to mediate cell death in the nervous system is based upon these observations.

2.2. Poly(ADP-ribosyl)ation and apoptosis

The cleavage of PARP-1 by caspases activated early in apoptosis provides one of the most recognizable observations of the role of poly(ADP-ribosyl)ation in cell death [6]. In fact, the presence of cleaved PARP-1 is one of the most utilized diagnostics for the detection of apoptosis in many cell types. The significance of this event, which produces 24 and 89 kDa fragments of PARP-1, is not fully understood. The 24 kDa fragment, which contains the DNA binding domain, possibly facilitates the apoptotic process by blocking access of DNA repair enzymes to the fragmented chromatin [7], while the 89 kDa fragment, which contains the automodification and catalytic domains, may be incapable of activation by DNA nicks, which suggests a possible mechanism to prevent energy depletion or DNA damaging signaling. Therefore, poly(ADP-ribosyl)ation, as mediated through PARP-1, plays an identifiable, but unknown, role in apoptotic cell death.

2.3. Poly(ADP-ribosyl)ation and necrosis

Poly(ADP-ribosyl)ation is a potentially very energetically expensive process. The synthesis of NAD^+, the substrate of PARP, is ATP-dependent. Further, the oxidoreduction capacity of this coenzyme is required in the electron transport chain in the mitochondria to maintain its proton gradient and thereby generate ATP. Because PARP-1 responds to DNA damage in a dose-dependent fashion, it is possible that highly activated PARP-1 can lead to the depletion of cellular energy stores following severe stress, which would lead to the loss of all energy-dependent cellular function, thereby initiating necrosis [8]. This NAD^+-depleting activity of PARP-1 was long thought to be its sole mechanism of eliciting cell death following cell stress.

3. PARP-1 and cell death in the nervous system

3.1. Excitotoxic neuronal cell death

The damage following stroke is manifest through both primary and secondary neurotoxic events. Primary neuronal injury occurs via oxygen and nutrient deprivation, while secondary injury ensues due to metabolic dysfunction and oxidative stress [9]. This secondary injury is thought to be the principal cause of the injury and subsequent morbidity following stroke. Coincidentally, excitotoxicity is in turn thought to be a major trigger for this neuronal injury. One pathway that is involved in excitotoxic cell death is DNA damage secondary to peroxynitrite formation. Glutamate is a major mediator of this secondary neuronal damage, in large part through the activation of *N*-methyl-D-aspartate (NMDA) receptors [10]. This results in increases in intracellular calcium and activation of calcium-dependent enzymes, in particular nitric oxide synthase (NOS). Activation of NOS results in the synthesis of nitric oxide (NO) and elevated levels of superoxide anion. The generation of NO and superoxide plays a key role in the excitotoxic damage induced by focal ischemia [11]. In addition to their own intrinsic cell damaging capabilities, NO and superoxide combine to form the highly reactive