
Receptor/Regulatory Molecules Pattern Changes: Caspases in Autism Spectrum Disorders

Dario Siniscalco, Nicola Antonucci, Sabatino Maione, and
Laura de Magistris

Introduction

Autism Spectrum Disorders

Autism spectrum disorders (ASDs) are heterogeneous pervasive neurodevelopmental disorders (Fasano 2010; Kim et al. 2012). Some of the common characteristics of ASDs are dysfunctions in social interaction and communication skills and repetitive and stereotypic verbal and nonverbal behaviors (Toro et al. 2010; Williams et al. 2010). Detailed description of these enigmatic conditions will be provided in other chapters in this book. Briefly, ASDs are associated with several biochemical events: oxidative stress, endoplasmic reticulum stress, decreased methylation capacity, limited production of glutathione, mitochondrial dysfunction, intestinal dysbiosis, inflammation, increased toxic metal burden, impaired detoxification, and immune dysregulation and immune activation of neuroglial cells (Ming et al. 2008; Bradstreet et al. 2010). Recently, it has been proposed a role for calcium signaling in

D. Siniscalco (✉)

Division of Pharmacology, Department of Experimental Medicine, Second University of Naples, Naples, Italy

e-mail: dariosin@uab.edu; taodar@yahoo.it

N. Antonucci

Biomedical Centre for Autism Research and Treatment, Bari, Italy

e-mail: info@antonucci.eu; dr.nicola.antonucci@gmail.com

S. Maione

Department of Experimental Medicine, Second University of Naples, Naples, Italy

e-mail: sabatino.maione@unina2.it

L. de Magistris

Department Magrassi-Lanzara and Centro Interuniversitario per ricerche su alimenti, nutrizione e apparato digerente (CIRANAD), Second University of Naples, Naples, Italy

e-mail: laura.demagistris@unina2.it

the pathogenesis of ASDs (Napolioni et al. 2011). The exact aetiology of ASDs is still unknown, and a complex combination of genetic, environmental (i.e., air pollution, organophosphates, heavy metals), and immunological factors could participate in ASD triggering events (Persico and Bourgeron 2006; Herbert 2010; Toro et al. 2010).

Recent evidences indicate a key role of several caspases in ASDs (Siniscalco et al. 2012). Caspases, a family of aspartate-specific cysteine-dependent proteases, are able to mediate both innate and adaptive immune responses (Yazdi et al. 2010; van de Veerdonk et al. 2011) and play a critical role by acting as regulators of monocyte cell fate (Parihar et al. 2010). Immune abnormalities and altered immune responses in autism pathogenesis have been well reported (Gupta et al. 2010). In addition, inflammatory responses, where caspases are strictly related, are highly altered in ASDs. Caspase is also able to mediate the pro-inflammatory cytokine production. Pro-inflammatory cytokines could induce some of the behavioral symptoms of autism (Croonenberghs et al. 2002).

This chapter will focus on the role and function of several caspases involved in ASDs development and maintenance.

Caspases

Caspases are an evolutionarily conserved structurally related family of aspartate-specific cysteine-dependent proteases (Lamkanfi et al. 2002). The main role of their function is in apoptotic and inflammatory signaling pathways. Beyond apoptosis, recent data have elucidated their non-apoptotic functions, such as proteolysis and other non-proteolytic functions. Indeed, these proteases are pleiotropic enzymes, as they take a role also in cell differentiation, proliferation, as well as in activation and nuclear reprogramming pathways (Algeciras-Schimmich et al. 2002).

Based on their discovery and publication and also on their activity mechanism, caspases are mainly classified in two categories: regulatory, or upstream initiator, caspases and effector (downstream) caspases (Williams et al. 2006). Caspases formed by large prodomains containing related homotypic oligomerization motifs have regulatory functions (caspase-1, caspase-2, caspase-4, caspase-5, caspase-9, caspase-11, caspase-12). These large prodomain caspases are responsible to activate the short prodomain caspases (executioner or effector caspase-3, caspase-6, caspase-7, caspase-14) by proteolytic maturation. Indeed, caspases are synthesized as inactive proenzymes (zymogens) with a prodomain of variable length and then activated following cleavage at specific aspartate cleavage sites (Lamkanfi et al. 2008). Activated effector caspases are able to cleave a wide range of intracellular structural and regulatory proteins, leading to a set of biochemical changes in cell morphology and eventual cell death (Chang et al. 2003). Some regulatory caspases (i.e., caspase-12) can act as executioner caspase. Indeed, caspases can play a role in both inflammatory and apoptotic processes (i.e., caspase-11 and caspase-12).

In the apoptosis pathway, caspases are responsible of specific cleavage of a wide variety of substrates implicated in the regulatory and execution phases of apoptosis. The cell death is the final outcome of these caspase-mediated proteolytic cascades.

In the non-apoptotic events, caspases could become activated independently of – or without – an apoptotic stimuli, thus leading to the cleavage of a specific subset of substrates, such as cytokines, kinases, transcription factors, and polymerases (Lamkanfi et al. 2007).

Caspase-1

Historically, caspase-1 is the first one discovered and identified. It is the best-characterized inflammatory caspase. Its role is determinant in the developing inflammation, as it is able to cleave the inactive 31 kDa cytokine pro-IL-1 β to generate the active 17 kDa mature form of IL-1 β (Cohen 1997), the potent pro-inflammatory cytokine. Caspase-1 is formed by two domains of 20 kDa and 10 kDa, respectively (Fig. 1). These subunits are derived from autoproteolysis of a large prodomain. This autocatalytic process is mediated by the interaction of the prodomain of the initiator caspase with its adaptor protein. Oligomerization of caspase-1 is required for auto-processing. Activation of caspase-1 requires the inflammasome formation, an activating molecular platform of a multiprotein complex. The major functions of inflammasome are recognizing cytosolic pathogen-associated molecular patterns and reacting to these patterns through activation of pro-inflammatory cytokines and thereby initiating inflammation (Fig. 2) (Salskov-Iversen et al. 2011). The active Nod-like receptor Nalp3 complexed to the apoptosis-associated protein (ASC) interacts with the caspase-1 forming the inflammasome protein scaffold and inducing the proteolytic caspase activity of the enzyme (Nicholas et al. 2011). In the inflammasome complex, caspase-1 is brought into close proximity with another caspase, caspase-5, to facilitate the cross activation (Martinon and Tschopp 2007). Once activated, caspase-1 subsequently cleaves the pro-IL-1 β . Besides IL-1 β , caspase-1 is responsible to process another pro-inflammatory cytokine, IL-18, also known as interferon- γ -inducing factor (Gracie et al. 2003; Bauernfeind et al. 2011). Interestingly, two mechanisms have been proposed to trigger activation of the inflammasome complex, one as a consequence of a bacterial product and the other following changes in the intracellular ionic environment. Interleukin-18 (IL-18) is also involved in regulating innate and acquired immune responses. IL-18 is expressed at sites of chronic inflammation, in autoimmune diseases, and in numerous infectious diseases (Gracie et al. 2003).

Through cytokine production, caspase-1 is able to trigger innate immune responses; the release of IL-1 β and IL-18 initiates Th1- and Th17-mediated adaptive immune responses (van de Veerdonk et al. 2011). Immune system takes an important role in ASD pathogenesis. Innate and adaptive immunity in ASDs shows several changes (Gupta et al. 2010). In addition, in the brain of autistic patients, innate and adaptive immune responses are increased through the Th1- pathway (Li et al. 2009). Autistic children show increased activation of both Th1- and Th2-mediated immune responses in peripheral blood mononuclear cells (PBMCs), together with an imbalance in the critical components of innate immunity, i.e., CD3+, CD4+, and CD8+ T cells, as well as in natural killer (NK) and in antigen-presenting (APC) cells (Gupta et al. 2010). Indeed, monocytic cells from autistic children show

Fig. 1 Structure of the caspase-1 protein (Reproduced from Worldwide Protein Data Bank (www.pdb.org). Copyright (c) 2012, Siniscalco. Permission is granted to copy, distribute, and/or modify this document under the terms of the GNU Free Documentation License)

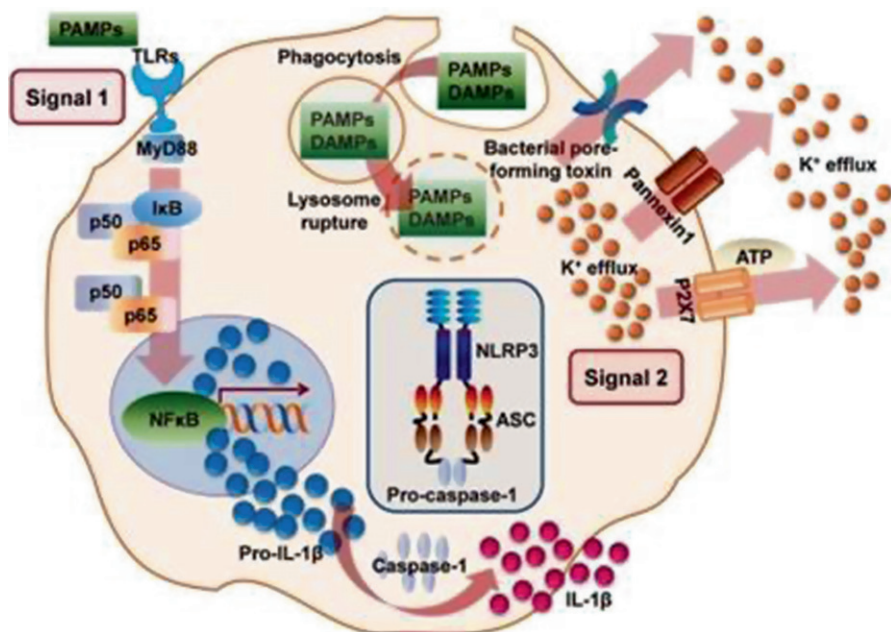
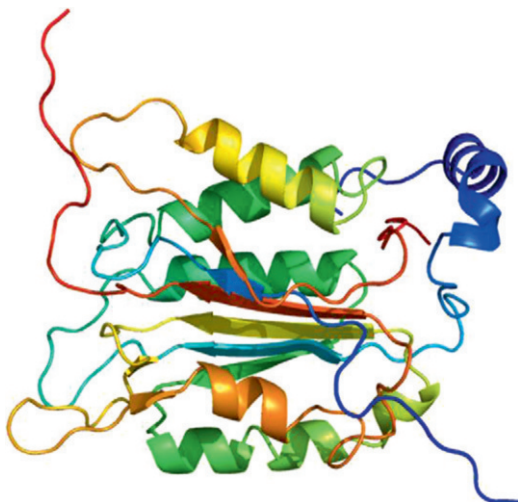


Fig. 2 A model for inflammasome activation. Several stimuli are able to trigger activation of NLRP3 leading to the secretion of interleukin (IL)-1β and IL-18. Signal 1 pathway, through the Toll-like receptor (TLR)-MyD88-NFκB signaling pathway, leads to the expression of the pro-IL-1β gene and production of the pro-IL-1β protein. Signal 2 pathway is responsible for the assembly of a large macromolecular complex, through the recruitment of the apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (CARD) (ASC) adaptor protein and pro-caspase-1 to NLRP3. Once activated, caspase-1 is able to cleave pro-IL-1β protein, thus forming the active IL-1 β interleukin (Reproduced and adapted from Yang et al. 2012)

excessive innate and adaptive immune responses (Jyonouchi et al. 2001). These cells are able to overproduce IL-1 β , resulting in long-term immune alterations (Enstrom et al. 2010). Caspase-1 gene is overexpressed in ASD-PBMCs indicating that this caspase is responsible for triggering immune response changes through cytokine production.

Caspase-2

Caspase-2, the most conserved member of the caspase family, is cleaved early during the apoptotic process. However, caspase-2 plays important functions in a number of stress-induced cell death pathways and in cell cycle maintenance, regulation, and DNA repair (Bouchier-Hayes 2010). Indeed, new mechanisms regulating caspase-2 activation have been proposed, suggesting that its regulation involves several kinases. This enzyme is linked to p53 via the activating complex PIDDosome (Vakifahmetoglu-Norberg and Zhivotovsky 2010). The caspase recruitment domain (CARD) of the pro-caspase-2 interacts with several proteins to form the complex PIDDosome. Probably, the assembly of the PIDDosome is engaged in response to DNA damage. After DNA damage, caspase-2 is rapidly phosphorylated. The phosphorylation represents a further type of caspase-2 modification that regulates its activity and specific functions. Beyond its roles in cell death processes, caspase-2 takes also part in the complex formed by RIP1 and TRAF2 that activates both the NF- κ B and p38 MAPK pathways (Lamkanfi et al. 2005). All these data highlight a complex regulation mechanism of caspase-2 activation and function. This regulation is strictly dependent upon the cell lineage, developmental stage, tissue type, and nature of the apoptotic stimulus. Caspase-2 is involved in the cellular stress response pathways, where through FoxO transcription factors, it regulates the oxidative stress response (Shalini et al. 2012). Moreover, unlike other caspases, caspase-2 is also constitutively localized in the nucleus, where it is involved in the G2/M DNA damage checkpoint maintenance (Shi et al. 2009). This role could be a novel biochemical process in ASDs. Indeed, metabolic abnormalities in ASDs have been confirmed (Ghanizadeh 2012). The “four major areas” involve immune dysregulation or inflammation, oxidative stress, mitochondrial dysfunction, and environmental toxicant exposures (Rossignol and Frye 2012). Oxidative stress-induced mechanisms are believed to be the major cause for ASD pathogenesis. Oxidative stress markers are strongly associated with major cellular damages and severe mitochondrial dysfunction in autistic pathology (Essa et al. 2011). Caspase-2 could be enrolled in the molecular processes triggering oxidative stress in ASDs, even if no specific intracellular protein substrates of caspase-2 have been identified so far. Prolonged endoplasmic reticulum (ER) stress (alterations in Ca²⁺ homeostasis and accumulation of unfolded proteins) triggers the activation of caspase-2 and ultimately results in caspase-mediated cell death (Murakami et al. 2007). Indeed, activated caspase-2 acts as sensors and/or effectors of cellular damage (Ruiz-Vela et al. 2005). In ASDs, the activation of caspase-2 could indicate possible cellular stress events.

Caspase-4 and Caspase-5

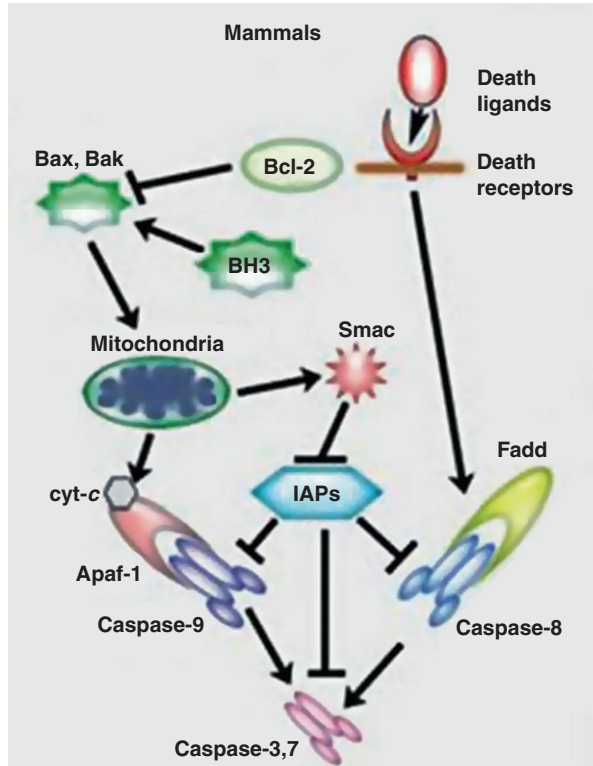
Caspase-4 and caspase-5 are members of the caspase-1 subfamily (inflammatory caspases) (Cohen 1997). However, they have different substrates with respect to caspase-1. Caspase-4 and caspase-5 arise from the gene duplication of a caspase-11-like ancestral gene (Martinon and Tschoop 2004). It has been proposed that both caspase-4 and caspase-5 could also act as upstream activator of caspase-1 (Faucheu et al. 1995; Martinon and Tschoop 2007). Recently, it has been reported that caspase-4 and caspase-5 are involved in cytokine maturation (Agard et al. 2010; Siniscalco et al. 2012). Indeed, caspase-4 is responsible to product IL-18 through TNF receptor-associated factor 6 interaction (Lakshmanan and Porter 2007). In this way, caspase-4 plays an important role in the LPS-induced TLR4-signaling pathway that leads to NF- κ B activation and consequent upregulation and secretion of IL-8 and CCL4. Caspase-4 and caspase-5 mRNAs are highly increased in ASD-PBMCs (Siniscalco et al. 2012). Both caspases, together with caspase-1, are believed to act as inducers of altered immune responses in autistic children. This fact is a novel issue opening the way for a possible new therapeutic strategy in ASDs. Blocking caspase activity could restore the immune imbalance in autistic children.

Caspase-3 and Caspase-7

Caspase-3 and caspase-7 belong to the executioner family and are closely related to the apoptotic process. They take a pivotal role during apoptosis. Upon initiation of the cell death program, caspase-3 and caspase-7 are recruited in the death-inducing signaling complex (DISC) and the apoptosome, respectively, through interaction with large prodomains of caspase-8 and caspase-9 (Lamkanfi et al. 2008) (Fig. 3). Once activated via proximity-induced activation, these caspases-8 and caspase-9 are able to induce an apoptotic cascade by proteolytically removing the linker region between the large and small catalytic subunits of caspase-3 and caspase-7 (Lamkanfi et al. 2008). Active caspase-3 and caspase-7 cleave several substrates, resulting in the morphological and biochemical hallmarks of apoptosis (Timmer and Salvesen 2007); however, they have different enzymatic sites, as well as a different pattern of cleaved substrates (Riedl et al. 2001).

Besides programmed cell death, they could also play physiological roles (Siniscalco et al. 2008). In inflammatory pathways, caspase-7 is a direct substrate of caspase-1 (Lamkanfi et al. 2008). In ASDs, it is noteworthy to consider that activated caspase-3 and caspase-7 are required for the proliferation of T lymphocytes (Adam-Klages et al. 2005; Paulsen et al. 2008; McComb et al. 2010; Siniscalco et al. 2012). It has been proposed that the caspase-mediated signaling pathway is involved in activation processes, such as NF- κ B- and AP-1-dependent gene transcription, leading to secretion of the autocrine growth factor IL-2 (Adam-Klages et al. 2005). This cytokine is able to enhance T-cell proliferation. Activated caspases are present in proliferating T cell in response to a strong stimulus that requires fast and extensive T-cell activation. When acting as activator of T lymphocyte proliferation, caspase-3 and caspase-7 are localized into the cytoplasm. Subcellular localization of caspases could indicate which kind of

Fig. 3 Caspase-dependent cell death machinery. In apoptotic pathway, death stimuli trigger activation of caspase-8 and caspase-9, which in turn are able to activate the executioner caspase-3 and caspase-7 (Reproduced and adapted from Kuranaga 2012)



activation process they are performing (Hayashi et al. 2006). Executioner caspases target proteins, such as PARP, that are localized in the nucleus; thus, they cleave nuclear substrates during apoptosis process, whereas cytoplasm-localized activated caspase-3 and caspase-7 are required for the proliferation of T lymphocytes.

Caspase-3 and caspase-7 take an important role in T lymphocyte activation in ASDs as they are responsible for triggering immune response imbalance. Indeed, both these caspases are highly increased and activated in ASD pathology (Figs. 4 and 5) (Siniscalco et al. 2012). However, a kind of executioner caspase-mediated mechanism of regulation has been proposed in the expansion phase of the T lymphocyte response. Here executioner caspases are activated in order to ensure the fast and efficient apoptosis of the activated T cells and in this way the successful termination of the immune response. The intensity of the stimuli could switch between the two models of actions (Adam-Klages et al. 2005).

Caspase-12

Caspase-12 belongs to the same caspase group of caspase-1, caspase-4, and caspase-5, the group I family (Szegezdi et al. 2003). Caspase-12 is a central protease in ER stress. The folding and maturation of proteins destined to the plasma membrane, extracellular space, and the exo/endocytic compartments is

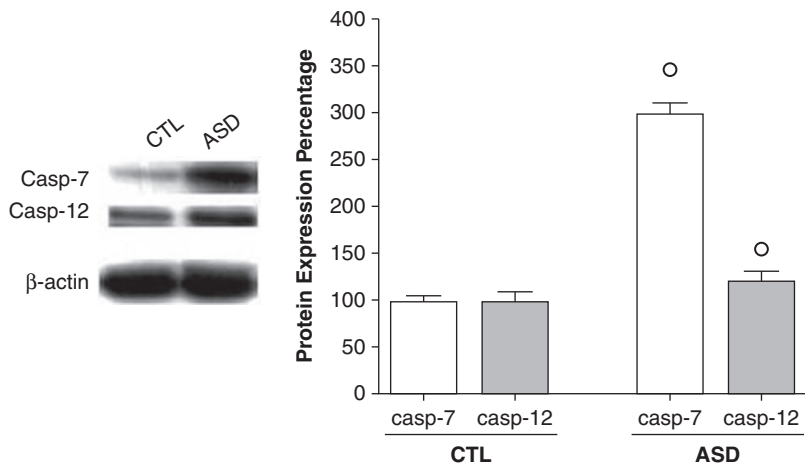


Fig. 4 Representative Western blot analysis of caspase-7 and caspase-12 protein levels in the PBMCs obtained from the ASD patients and the healthy controls, respectively. Legend: *CTL* healthy control subjects; *ASD* autism spectrum disorder patients. The histograms indicate percentage variations in caspase-7 and caspase-12 protein levels normalized with respect to the signal obtained for β -actin housekeeping protein in the PBMCs of ASD patients compared to the healthy controls (CTL). $^{\circ}$ indicates significant differences vs healthy subjects. $P < 0.05$ was considered as the level of significance (Data were reported in Siniscalco et al. 2012)

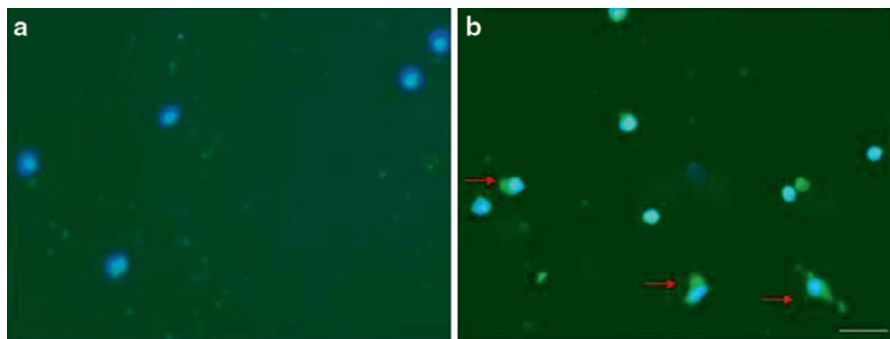


Fig. 5 Representative fluorescent photomicrograph of PBMCs showing immunocytochemistry for active caspase-3. *Arrows* indicate active form caspase-3-positive staining (green fluorescent). Cell nuclei were counterstained with bisbenzimidazole (blue fluorescence). **a** healthy control subjects; **b** autism spectrum disorder patients. Scale bar: 15 μ m (Data were reported in Siniscalco et al. 2012)

performed in ER (Zhang and Kaufman 2006). The ER stress is a complex molecular mechanism induced by accumulation of unfolded protein aggregates. ER imbalance in Ca^{2+} homeostasis and/or in the protein-folding machinery triggers activation of caspase-12 (Momoi 2004). Indeed, caspase-12 is localized

to the ER and activated by ER stress. It is noteworthy to consider that ASDs are related to endoplasmic reticulum (ER) stress (Fujita et al. 2010). Indeed, in ASDs ER stress arises from mutations in genes encoding synaptic adhesion molecules, triggers a trafficking disorder of synaptic receptors, and leads to their impaired synaptic function and signal transduction (Momoi et al. 2009). Caspase-12 plays a pivotal role in these molecular events in ASD pathophysiology (Siniscalco et al. 2012).

Activated caspase-12 can act as a regulator of inflammatory caspase-1-mediated signaling pathways and as sensor and/or effector of cellular damage (Ruiz-Vela et al. 2005). According to this hypothesis, caspase-12 takes a role in regulation and blocking the inflammatory and innate immune responses initiated by caspase-1, thus predisposing the organism to severe sepsis, protecting the organism from possible damaging effects (Scott and Saleh 2007).

Conclusion

On the light of the several biochemical pathways where caspases are enrolled in ASDs (Fig. 6), caspases could be an interesting potential target to better define the immune and inflammatory molecular pathways involved in autism

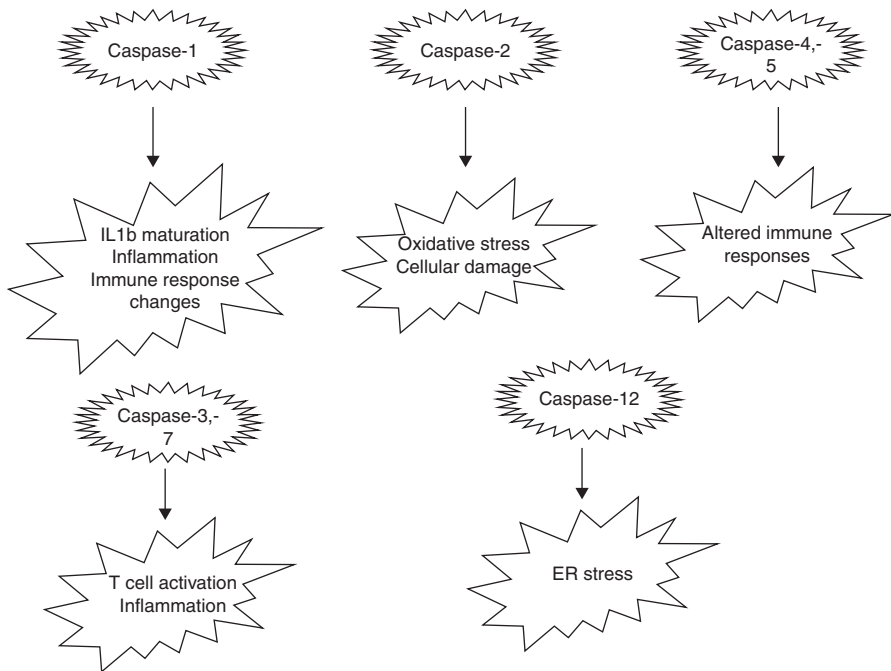


Fig. 6 Proposed model of caspase involvement in ASDs

pathophysiology. Validation of caspases as a diagnostic tool and possible therapeutic target in clinical setting is the next expectation of ASD research.

Key Terms

Adaptive immune system. This term is referred to highly specialized cells and processes that eliminate or prevent pathogenic growth.

Apoptosis. This term derives from an ancient Greek word which means “the falling of leaves from a tree.” It indicates a form of programmed cell death, a complex mix of biochemical changes and activations that provide cell death. Specific features are condensation of the nuclei, DNA fragmentation, chromatin condensation, generation of involuted membrane segments, and cellular shrinkage mitochondria disintegration.

Caspases. Caspases are a structurally related family of aspartate-specific cysteine-dependent proteases. They are involved in the regulatory and execution phases of several biochemical events.

Cytokines. Small signaling molecules secreted by several cell types in order to communicate with other cells.

Inflammasome. A complex multiprotein oligomer consisting, at least, of caspase-1, caspase-5, apoptosis-associated speck-like protein containing a CARD, NOD-like receptor, and apoptosis-associated protein. It is assembled for activation of inflammatory processes.

Innate immune system. The innate immune system comprises cells and mechanisms that defend the host from infections by other organisms in a nonspecific manner.

Oxidative stress. It is an imbalance between the production of reactive oxygen species and the mechanism of detoxification of a cell.

Proteases. Proteases are enzymes that catalyze proteolysis, the reactions of hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein.

Key Facts of Caspases

- Caspases are necessary for proteolytic activation of several biochemical pathways.
- Usually, they are related to apoptotic functions; however, several caspase-mediated non-apoptotic pathways have been elucidated.
- By cleaving a specific subset of substrates, caspases are responsible to trigger a cascade of molecular activations.
- Inflammatory events, both innate and adaptive immune responses, are molecular pathways in which caspases are involved.
- Several diseases and pathological states show caspase upregulation or over-activation.

Summary Points

- This chapter focuses on caspases involvement in autism.
- Caspases are proteolytic enzymes involved in many activation processes.
- Among the molecular pathway in which caspases take a pivotal role, inflammation and both innate and adaptive immune responses are mainly susceptible of caspase action.
- Autism spectrum disorders show several biochemical and cellular changes. Immune abnormalities and altered immune responses in autism have been reported.
- Recently, upregulation and over-activation of several caspase have been demonstrated in autistic children.
- Caspases could be an interesting target to better unravel the immune and inflammatory molecular pathways involved in autism pathophysiology.

References

- Adam-Klages S, Adam D, Janssen O, Kabelitz D. Death receptors and caspases: role in lymphocyte proliferation, cell death, and autoimmunity. *Immunol Res.* 2005;33(2):149–66.
- Agard NJ, Maltby D, Wells JA. Inflammatory stimuli regulate caspase substrate profiles. *Mol Cell Proteomics.* 2010;9(5):880–93.
- Algeciras-Schimmich A, Barnhart BC, Peter ME. Apoptosis independent functions of killer caspases. *Curr Opin Cell Biol.* 2002;14:721–6.
- Bauernfeind F, Ablasser A, Bartok E, Kim S, Schmid-Burgk J, Cavlar T, et al. Inflammasomes: current understanding and open questions. *Cell Mol Life Sci.* 2011;68(5):765–83.
- Bouchier-Hayes L. The role of caspase-2 in stress-induced apoptosis. *J Cell Mol Med.* 2010;14(6A):1212–24.
- Bradstreet JJ, Smith S, Baral M, Rossignol DA. Biomarker-guided interventions of clinically relevant conditions associated with autism spectrum disorders and attention deficit hyperactivity disorder. *Altern Med Rev.* 2010;15:15–32.
- Chang DW, Ditsworth D, Liu H, Srinivasula SM, Alnemri ES, Yang X. Oligomerization is a general mechanism for the activation of apoptosis initiator and inflammatory procaspases. *J Biol Chem.* 2003;278(19):16466–9.
- Cohen GM. Caspases: the executioners of apoptosis. *Biochem J.* 1997;326(Pt 1):1–16.
- Croonenberghs J, Bosmans E, Deboutte D, Kenis G, Maes M. Activation of the inflammatory response system in autism. *Neuropsychobiology.* 2002;45:1–6.
- Enstrom AM, Onore CE, Van de Water JA, Ashwood P. Differential monocyte responses to TLR ligands in children with autism spectrum disorders. *Brain Behav Immun.* 2010; 24(1):64–71.
- Essa MM, Braidy N, Vijayan KR, Subash S, Guillemin GJ. Excitotoxicity in the pathogenesis of autism. *Neurotox Res.* 2013; 23(4):393–400.
- Fasano A. Intestine, leaky gut, autism and probiotics. In: *Cutting edge therapies.* Lyndhurst: Barnes & Noble; 2010. p. 192–198.
- Faucheu C, Diu A, Chan AW, Blanchet AM, Miossec C, Hervé F, et al. A novel human protease similar to the interleukin-1 beta converting enzyme induces apoptosis in transfected cells. *EMBO J.* 1995;14(9):1914–22.
- Fujita E, Dai H, Tanabe Y, Zhiling Y, Yamagata T, Miyakawa T, et al. Autism spectrum disorder is related to endoplasmic reticulum stress induced by mutations in the synaptic cell adhesion molecule, CADM1. *Cell Death Dis.* 2010;1(6):e47.

- Ghanizadeh A. Malondialdehyde, Bcl-2, superoxide dismutase and glutathione peroxidase may mediate the association of sonic hedgehog protein and oxidative stress in autism. *Neurochem Res.* 2012;37(4):899–901.
- Gracie JA, Robertson SE, McInnes IB. Interleukin-18. *J Leukoc Biol.* 2003;73(2):213–24.
- Gupta S, Samra D, Agrawal S. Adaptive and innate immune responses in autism: rationale for therapeutic use of intravenous immunoglobulin. *J Clin Immunol.* 2010;30:S90–6.
- Hayashi N, Shirakura H, Uehara T, Nomura Y. Relationship between SUMO-1 modification of caspase-7 and its nuclear localization in human neuronal cells. *Neurosci Lett.* 2006;397(1–2):5–9.
- Herbert MR. Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders. *Curr Opin Neurol.* 2010;23:103–10.
- Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol.* 2001;120(1–2):170–9.
- Kim KY, Jung YW, Sullivan GJ, Chung L, Park IH. Cellular reprogramming: a novel tool for investigating autism spectrum disorders. *Trends Mol Med.* 2012; 18(8):463–471.
- Kuranaga E. Beyond apoptosis: caspase regulatory mechanisms and functions in vivo. *Genes Cells.* 2012; in press, doi:10.1111/j.1365-2443.2011.01579.x
- Lakshmanan U, Porter AG. Caspase-4 interacts with TNF receptor-associated factor 6 and mediates lipopolysaccharide-induced NF- κ B-dependent production of IL-8 and CC chemokine ligand 4 (macrophage-inflammatory protein-1). *J Immunol.* 2007;179(12):8480–90.
- Lamkanfi M, Declercq W, Kalai M, Saelens X, Vandenabeele P. Alice in caspase land. A phylogenetic analysis of caspases from worm to man. *Cell Death Differ.* 2002;9:358–61.
- Lamkanfi M, D'hondt K, Vande Walle L, van Gorp M, Denecker G, Demeulemeester J, et al. A novel caspase-2 complex containing TRAF2 and RIP1. *J Biol Chem.* 2005;280(8):6923–32.
- Lamkanfi M, Festjens N, Declercq W, Vanden Berghe T, Vandenabeele P. Caspases in cell survival, proliferation and differentiation. *Cell Death Differ.* 2007;14(1):44–55.
- Lamkanfi M, Kanneganti TD, Van Damme P, Vanden Berghe T, Vanoverberghe I, Vandekerckhove J, et al. Targeted peptidocentric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. *Mol Cell Proteomics.* 2008;7(12):2350–63.
- Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, et al. Elevated immune response in the brain of autistic patients. *J Neuroimmunol.* 2009;207(1–2):111–6.
- Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ.* 2007;14(1):10–22.
- Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell.* 2004;117(5):561–74.
- McComb S, Mulligan R, Sad S. Caspase-3 is transiently activated without cell death during early antigen driven expansion of CD8(+) T cells in vivo. *PLoS One.* 2010;5(12):e15328.
- Ming X, Brimacombe M, Chaaban J, Zimmerman-Bier B, Wagner GC. Autism spectrum disorders: concurrent clinical disorders. *J Child Neurol.* 2008;23:6–13.
- Momoi T, Fujita E, Senoo H, Momoi M. Genetic factors and epigenetic factors for autism: endoplasmic reticulum stress and impaired synaptic function. *Cell Biol Int.* 2009;34(1):13–9.
- Momoi T. Caspases involved in ER stress-mediated cell death. *J Chem Neuroanat.* 2004;28(1–2):101–5.
- Murakami Y, Aizu-Yokota E, Sonoda Y, Ohta S, Kasahara T. Suppression of endoplasmic reticulum stress-induced caspase activation and cell death by the overexpression of Bcl-xL or Bcl-2. *J Biochem.* 2007;141(3):401–10.
- Napolioni V, Persico AM, Porcelli V, Palmieri L. The mitochondrial aspartate/glutamate carrier AGC1 and calcium homeostasis: physiological links and abnormalities in autism. *Mol Neurobiol.* 2011;44(1):83–92.
- Nicholas SA, Bubnov VV, Yasinska IM, Sumbayev VV. Involvement of xanthine oxidase and hypoxia-inducible factor 1 in Toll-like receptor 7/8-mediated activation of caspase 1 and interleukin-1 β . *Cell Mol Life Sci.* 2011;68(1):151–8.

- Parihar A, Eubank TD, Doseff AI. Monocytes and macrophages regulate immunity through dynamic networks of survival and cell death. *J Innate Immun.* 2010;2(3):204–15.
- Paulsen M, Ussat S, Jakob M, Scherer G, Lepenies I, Schütze S, et al. Interaction with XIAP prevents full caspase-3/-7 activation in proliferating human T lymphocytes. *Eur J Immunol.* 2008;38(7):1979–87.
- Persico AM, Bourgeron T. Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. *Trends Neurosci.* 2006;29:349–58.
- Riedl SJ, Fuentes-Prior P, Renatus M, Kairies N, Krapp S, Huber R, et al. Structural basis for the activation of human procaspase-7. *Proc Natl Acad Sci USA.* 2001;98:14790–5.
- Rossignol DA, Frye RE. A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. *Mol Psychiatry.* 2012;(4):389–401.
- Ruiz-Vela A, Opferman JT, Cheng EH, Korsmeyer SJ. Proapoptotic BAX and BAK control multiple initiator caspases. *EMBO Rep.* 2005;6(4):379–85.
- Salskov-Iversen ML, Johansen C, Kragballe K, Iversen L. Caspase-5 expression is upregulated in lesional psoriatic skin. *J Invest Dermatol.* 2011;131(3):670–6.
- Scott AM, Saleh M. The inflammatory caspases: guardians against infections and sepsis. *Cell Death Differ.* 2007;14(1):23–31.
- Shalini S, Dorstyn L, Wilson C, Puccini J, Ho L, Kumar S. Impaired antioxidant defence and accumulation of oxidative stress in caspase-2-deficient mice. *Cell Death Differ.* 2012;19(8):1370–1380.
- Shi M, Vivian CJ, Lee KJ, Ge C, Morotomi-Yano K, Manzl C, et al. DNA-PKcs-PIDDosome: a nuclear caspase-2-activating complex with role in G2/M checkpoint maintenance. *Cell.* 2009;136(3):508–20.
- Siniscalco D, Giordano C, Fuccio C, Luongo L, Ferraraccio F, Rossi F, et al. Involvement of subtype 1 metabotropic glutamate receptors in apoptosis and caspase-7 over-expression in spinal cord of neuropathic rats. *Pharmacol Res.* 2008;57(3):223–33.
- Siniscalco D, Sapone A, Giordano C, Cirillo A, de Novellis V, de Magistris L, et al. The expression of caspases is enhanced in peripheral blood mononuclear cells of autism spectrum disorder patients. *J Autism Dev Disord.* 2012;42(7):1403–10.
- Szegezdi E, Fitzgerald U, Samali A. Caspase-12 and ER-stress-mediated apoptosis: the story so far. *Ann N Y Acad Sci.* 2003;1010:186–94.
- Timmer JC, Salvesen GS. Caspase substrates. *Cell Death Differ.* 2007;14(1):66–72.
- Toro R, Konyukh M, Delorme R, Leblond C, Chaste P, Fauchereau F, et al. Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. *Trends Genet.* 2010;26:363–72.
- Vakifahmetoglu-Norberg H, Zhivotovsky B. The unpredictable caspase-2: what can it do? *Trends Cell Biol.* 2010;20(3):150–9.
- van de Veerdonk FL, Netea MG, Dinarello CA, Joosten LA. Inflammasome activation and IL-1 β and IL-18 processing during infection. *Trends Immunol.* 2011;32(3):110–6.
- Williams DW, Kondo S, Krzyzanowska A, Hiromi Y, Truman JW. Local caspase activity directs engulfment of dendrites during pruning. *Nat Neurosci.* 2006;9(10):1234–6.
- Williams K, Wheeler DM, Silove N, Hazell P. Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). *Cochrane Database Syst Rev.* 2010; 8:CD004677. doi: 10.1002/14651858.CD004677.pub2.
- Yang CS, Shin DM, Jo EK. The role of NLR-related protein 3 inflammasome in host defense and inflammatory diseases. *Int Neurobiol J.* 2012;16:2–12.
- Yazdi AS, Guarda G, D’Ombrian MC, Drexler SK. Inflammatory caspases in innate immunity and inflammation. *J Innate Immun.* 2010;2(3):228–37.
- Zhang K, Kaufman RJ. The unfolded protein response: a stress signaling pathway critical for health and disease. *Neurology.* 2006;66(2 Suppl 1):S102–109.