Int J Biol Markers 2012; 27 (1): 60-63
DOI: 10.5301/JBM.2012.8991

CASE REPORT

Three apoptotic genes are upregulated in a patient with Alzheimer's disease and well-differentiated squamous cell carcinoma

Michele Salemi[1,2], Domenica Giuffrida[1], Maria C. Giuffrida[1,4], Pier Franco Soma[3], Sandro La Vignera[1], Laura Cimino[1], Rosita A. Condorelli[1], Carmelo Romano[2], Paolo Bosco[2], Lucia O. Vicari[5], Aldo E. Calogero[1]

[1]Section of Endocrinology, Andrology and Internal Medicine, Department of Internal Medicine and Systemic Diseases, and Master in Andrological and Human Reproduction Sciences, University of Catania, Catania - Italy
[2]Laboratory of Cytogenetics, Oasi Institute for Research on Mental Retardation and Brain Aging, Troina - Italy
[3]Plastic Surgery and Burns Center, Cannizzaro Hospital, Catania - Italy
[4]Fondazione Fulvio Frisone, Catania - Italy
[5]Master in Clinical Experimental Medicine and Cell Physiopathology, University of Catania, Catania - Italy

ABSTRACT

We report the case of a 74-year-old man with Alzheimer's disease (AD) and an extensive ulcerative lesion on the right ear. AD is a neurodegenerative disease with progressive loss of memory and cognitive deterioration. It has been suggested that apoptotic cell injury and eventually cell death is a major contributor to the AD neurodegenerative process. The ulcerative lesion was surgically excised and the histological analysis reported a well-differentiated squamous cell carcinoma. Caspase-3 (*CASP3*) plays an important role in neuronal death during nervous system development and under certain pathological conditions. Furthermore, in vitro and in vivo studies reported elevated expression and activation of *CASP3* in models of AD. Molecular epidemiological studies suggest that *CASP3* may contribute to head and neck squamous cell carcinoma susceptibility and disease progression and that increased *CASP3* expression is associated with tumors of the head. Also poly (ADP-ribose) polymerase 1 (*PARP1*) and the leucine zipper downregulated in cancer 1 (*LDOC1*) genes play a proapoptotic role. We therefore evaluated the differential expression of *LDOC1*, PARP1, and *CASP3* mRNA in peripheral blood leukocytes of our patient. We found increased expression of all these genes compared with the expression in control subjects.

Key words: Alzheimer's disease, Squamous cell carcinoma, Apoptosis, *PARP1*, Caspase-3, *LDOC1*, qRT-PCR

Received: June 1, 2011; Accepted: October 28, 2011

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease with progressive loss of memory and deterioration of comprehensive cognition. It is characterized by senile plaques, neurofibrillary tangles, and extensive neuronal loss. Interestingly, patients with sporadic or familial AD share common clinical and neuropathological features (1). Amyloid beta (Aβ), which is generated by aberrant processing of the amyloid precursor protein (APP), is the principal constituent of senile plaques. Aβ peptides and APP mutants induce neurodegeneration characteristic of apoptosis (2, 3). Several studies (4, 5) have suggested that apoptotic cell injury, and eventually cell death, is a major contributor to the neurodegenerative process in AD.

Squamous cell carcinoma (SCC) is the second most common cancer of the skin occurring in the head and neck region and accounts for 20% of cutaneous malignancies (6). SCC frequently develops on the sun-exposed skin of middle-aged and elderly individuals (7). The lifetime risk of developing skin cancer has been estimated to be 29-55% for basal cell carcinoma (BCC) and 7-11% for SCC. Cutaneous SCC is known to be more aggressive and prone to metastasis than BCC (7, 8).

SCC of the external auditory canal is rare compared to SCC of other cutaneous sites (1 out of 5,000-15,000 persons with cancer) and is associated with high morbidity and mortality rates (9). Unfortunately, most of these tumors are diagnosed at a late stage because the initial symptoms are similar to those of other, benign diseases of the ear.

Apoptosis is a programmed cell death process that takes place under normal physiological and pathological conditions. Caspases, a family of cysteine-dependent aspartate-specific proteases, are important mediators of the apoptotic process (10). Among them, apoptosis-related cysteine peptidase 3 (*CASP3*; OMIM 600636), which maps to human chromosome 4q35, is involved in both extrinsic and intrinsic apoptotic pathways. In

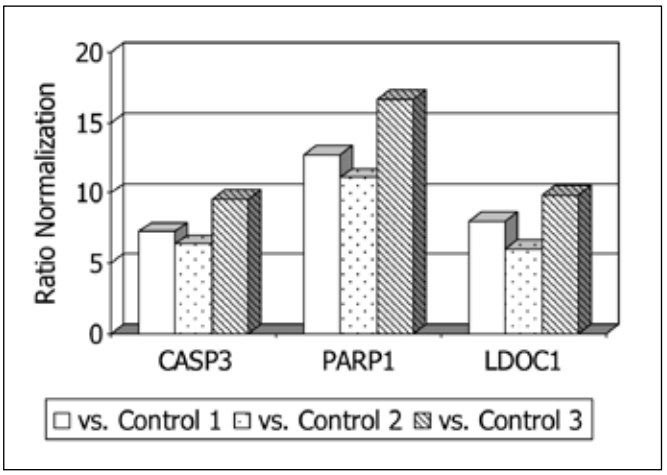

Fig. 1 - *LDOC1, PARP1 and CASP3 mRNA expression in case and controls.*

addition, *CASP3* plays an important role in neuronal death during nervous system development and under certain pathological conditions.

In vitro (11, 12) and in vivo (13, 14) studies have reported increased expression and activation of several caspases in AD models. Moreover, caspase levels are elevated in the brain of patients with severe definitive AD (4, 15, 16). Recent molecular epidemiological studies suggest that *CASP3* may contribute to head and neck SCC susceptibility and disease progression and that increased *CASP3* expression is associated with tumors of the head (17).

Yu and colleagues (18) showed that the activation of the poly (ADP-ribose) polymerase 1 (*PARP1*) gene (OMIM 173870) is required for translocation of the apoptosis-inducing factor (AIF) from the mitochondria to the nucleus (19). *PARP1* is proteolytically cleaved at the onset of apoptosis by *CASP3* (19); furthermore, *PARP1* activity and poly (ADP-ribose) (PAR) polymer mediate *PARP1*-induced cell death (19, 20). Genetic and pharmacological studies have demonstrated that *PARP1* overexpression is a key mediator of programmed-necrotic cell death in vivo, and *PARP1* appears to be also involved in programmed cell death processes as apoptosis (20-22).

In addition, the leucine zipper, downregulated in cancer 1 (*LDOC1*) gene (OMIM 300402), located at Xq27, encodes a 146-amino-acid-long LDOC1 nuclear protein which is highly expressed in the brain (23, 24). The Xq27 region is characterized by an elevated density of large segmental duplications. Because of the different orientation of segmental duplications, their recombinational interactions may result in deletion, duplications, and inversion of the *LDOC1*-containing genomic region (25).

CASE REPORT

A 74-year-old man was admitted to the Cannizzaro Hospital, Catania, Italy. He had been affected by AD for at least 10 years and was successfully treated with antipsychotic drugs including quetiapine. At the time he presented to our clinic, he was reported to have had a change in personality characterized by increased anger and irritability in addition to an impaired sense of direction with a tendency to get lost. Cognitive examination at the initial evaluation showed alert mentation with fluent speech. The patient was able to repeat names and follow commands. Professionally, he had experienced troubles over the previous few years which ultimately led to his retirement. He exhibited name-finding and word-finding difficulty. No hallucinations or delusions were reported. He did not smoke or use illegal drugs. No bladder or bowel incontinence was reported. The patient showed an extensive ulcerative lesion of approximately 4.5 cm on his right ear. In March 2011 he underwent surgical excision of the lesion and histological analysis revealed a well-differentiated SCC. The surgical margins were negative, no lymph nodes were removed, and there were no metastases to other areas of the skin.

We evaluated the possible differential expression of *CASP3, PARP1,* and *LDOC1* mRNA by quantitative real-time polymerase chain reaction (qRT-PCR) in the peripheral blood leukocytes of our patient and 3 healthy control men. RNA extraction from peripheral blood leukocytes was performed using the RNeasy Mini Kit

TABLE I - *LDOC1, PARP1* AND *CASP3* mRNA EXPRESSION IN CASE AND CONTROLS

	Case/Control 1				Case/Control 2				Case/Control 3			
	Control 1		Case		Control 2		Case		Control 3		Case	
	M. Cp.	RT	M. Cp.	RT	M. Cp.	RT	M. Cp.	RT	M. Cp.	RT	M. Cp.	RT
Target gene *LDOC1* expression	30.02	1.000	29.72	7.498	30.17	1.000	29.72	6.051	30.20	1.000	29.72	9.854
Target gene *PARP1* expression	29.15	1.000	26.09	12.69	27.41	1.000	26.09	11.09	27.45	1.000	26.09	16.68
Target gene *CASP3* expression	28.99	1.000	28.74	7.241	29.26	1.000	28.74	6.350	29.01	1.000	28.74	9.516
Reference gene *GAPDH* expression	26.92	-------	29.53	-------	27.38	-------	29.53	-------	26.53	-------	29.53	-------

M. Cp., mean crossing point; RT, ratio normalization

(QIAGEN Sciences, Germantown, PA, USA) according to the manufacturer's protocol. The RNA quality and quantity were checked by spectrophotometry. To avoid any genomic DNA contamination during qRT-PCR, a brief incubation of the samples at 42°C with a specific Wipeout buffer (QuantiTect Reverse Transcription Kit, QIAGEN Sciences) was carried out. Retro-transcription of 600 ng of total RNA from each sample was then performed in a final volume of 50 µL and the generated cDNA was used as a template for qRT-PCR analysis using gene expression products. For each sample, RT-PCR reactions were carried out in duplicate using 2.5 µL of cDNA and QuantiTect Probe PCR Master Mix (QIAGEN Sciences) in a total volume of 50 µL. The target gene (*CASP3, PARP1* and *LDOC1*) assays and the *GAPDH* reference gene assay were obtained from Applied Biosystems (Carlsbad, CA, USA). The thermal cycling conditions consisted of 1 cycle for 2 minutes at 50°C, 1 cycle of 15 minutes at 95°C, and 40 cycles for 15 seconds at 94°C followed by 1 minute at 60°C. Real-time analysis was performed on an ABI PRISM 5700 Sequence Detector (Applied Biosystems). The amplified transcripts were quantified using the comparative Ct method (26) and relative quantification analysis data were obtained using the comparative Delta-Delta Ct method included in the software version 1.3 supplied with the Applied Biosystems device. *CASP3*, *PARP1*, and *LDOC1* expression levels were normalized to the *GAPDH* expression level and target mean crossing point (Cp) definition was used to indicate the mean normalized cycle threshold.

DISCUSSION

We found increased expression of *CASP3*, *PARP1*, and *LDOC1* in the studied patient with AD and SCC of the external auditory canal compared with the 3 controls (Fig. 1, Tab. I). This finding suggests that *CASP3*-, *PARP1*-, and *LDOC1*-mediated cell death pathways are particularly active. In addition, the *CASP3* overexpression found in this patient confirmed previous studies showing an association between overexpression of this gene and tumors of the mouth (17). The overexpressed *PARP1* and *LDOC1* we found may be regarded as novel biomarkers for SCC of the external auditory canal and AD.

Increased caspase activity as well as cleavage of caspase substrates have also been detected in the AD brain (27, 28). Rohn et al (28) reported the activation of mitochondrial and receptor-mediated apoptotic pathways in hippocampal brain sections of patients with AD, where active caspase-9 was co-localized with active caspase-8. Moreover, it has been reported that neurotoxic insults induce APP elevation, followed by *CASP3* activation, which causes both apoptosis and the proteolytic processing of APP that leads to Aβ formation (29).

Interestingly, it has been suggested that *CASP3* may contribute to head and neck SCC susceptibility and disease progression (17). It is known that after the apoptotic stimulus, *CASP3* cleaves and activates *PARP1*, which triggers cell death. Thus, *CASP3* and *PARP1* belong to the same apoptotic pathway (18, 19). *PARP1* hyperactivation appears to be also a key mediator of cell death in low- or non-proliferating cells in vivo (20, 21). Uncontrolled poly-ADP-ribosylation reactions can result in massive necrotic cell death and tissue damage, which in turn often lead to severe inflammatory or neurodegenerative disorders (20, 21). Indeed, recent studies using DNA damaging agents such as N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), hydrogen peroxide (H_2O_2) or peroxynitrite, which are known to induce necrosis at high concentrations, showed that pharmacological inhibition of *PARP1* activity or knockout of the *PARP1* gene blocks programmed-necrotic cell death induced by these agents (20-22). Numerous reports indicate that inhibition or absence of *PARP1* provides remarkable protection in disease models such as septic shock, diabetes, stroke, myocardial infarction and ischemia, which are characterized predominantly by programmed-necrotic cell death (22).

In addition, *LDOC1* has been shown to inhibit the activation of NF-kappaB through ligand-induced stimulation by TNF-α or PMA in a dose-dependent manner, and viability studies demonstrated that TNF-α or PMA-induced antiproliferative effects were significantly enhanced by stable transfection of cells with *LDOC1* (24). These observations suggested that *LDOC1* is a novel regulator of NF-kappaB that can affect the PMA- or TNF-α-mediated pathway to apoptosis through inhibition of NF-kappaB (24). Furthermore, the transcription factor MZF-1 has been shown to interact with *LDOC1* and to enhance the apoptosis-inducing activity of *LDOC1* (30). Indeed, *LDOC1* overexpression causes externalization of the cell membrane phosphatidylserine, which is characteristic of early-phase apoptotic events, and reduced cell viability in some human cell lines (31). Mizutani et al (32) have shown that ectopically expressed *LDOC1* is localized to the nucleus and induces apoptosis, accompanied by an increase in the tumor p53 protein content but not in *TP53* transcription, suggesting that *LDOC1* inhibits the degradation of p53. Lynn et al (31) in an expression array study of male young-onset hypertension have suggested that innate immune response and cell-proliferation regulation may play important downstream roles in the development of hypertension and specifically that *LDOC1* plays a key role in the regulatory mechanisms related to apoptosis in hypertension.

In conclusion, the observed overexpression of *CASP3*, *PARP1*, and *LDOC1* confirmed the important role of apoptosis in both well-differentiated carcinoma without metastases and AD. Indeed, several findings suggest that the apoptotic mechanism is activated in neurodegenerative disease including AD. This finding needs to be confirmed in a greater number of patients.

Conflict of interest statement: The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Address for correspondence:
Dr. Michele Salemi
Oasi Istitute for Research on Mental Retardation and Brain Aging
94018 Troina (Enna), Italy
e-mail: micezia@tiscali.it

REFERENCES

1. Shimohama S. Apoptosis in Alzheimer's disease-an update. Apoptosis 2000; 5: 9-16.
2. Loo DT, Copani A, Pike CJ, Whittemore ER, Walencewicz AJ, Cotman CW. Apoptosis is induced by beta amyloid in cultured central nervous system neurons. Proc Natl Acad Sci USA 1993; 90: 7951-5.
3. Zhao B, Chrest FJ, Horton WE Jr, Sisodia SS, Kusiak JW. Expression of mutant amyloid precursor proteins induces apoptosis in PC12 cells. J Neurosci Res 1997; 47: 253-63.
4. Raina AK, Hochman A, Zhu X, et al. Abortive apoptosis in Alzheimer's disease. Acta Neuropathol 2001; 101: 305-10.
5. Cohen GM. Caspases: the executioners of apoptosis. Biochem J 1997; 326: 1-16.
6. Clayman GL, Lee JJ, Holsinger FC, et al. Mortality risk from squamous cell skin cancer. J Clin Oncol 2005; 23: 759-65.
7. Miller DL, Weinstock MA. Non melanoma skin cancer in the United States: incidence. J Am Acad Dermatol 1994; 30: 774-8.
8. Epstein E, Epstein NN, Bragg K, Linden G. Metastases from squamous cell carcinoma of the skin. Arch Dermatol 1968; 97: 245-51.
9. Crabtree JA, Britton BH, Pierce MK. Carcinoma of the external auditory canal. Laryngoscope 1976; 86: 405-15.
10. Thornberry NA, Lazebnik Y. Caspases: enemies within. Science 1998; 281: 1312-6.
11. Jordan J, Galindo MF, Miller RJ. Role of calpain and interleukin-1beta converting enzyme-like proteases in the beta-amyloid–induced death of rat hippocampal neurons in culture. J Neurochem 1997; 68: 1612-21.
12. Nakagawa T, Zhu H, Morishima N, et al. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-b. Nature 2000; 403: 98-103.
13. LaFerla FM, Tinkle BT, Bieberich CJ, Haudenschild CC, Jay G. The Alzheimer's A beta peptide induces neurodegeneration and apoptotic cell death in transgenic mice. Nat Genet 1995; 9: 21-30.
14. Sheng JG, Jones RA, Zhou XQ, et al. Interleukin-1 promotion of MAPK-p38 overexpression in experimental animals and in Alzheimer's disease: potential significance for tau protein phosphorylation. Neurochem Int 2001; 39: 341-8.
15. Stadelmann C, Deckwerth TL, Srinivasan A, et al. Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease: evidence for apoptotic cell death. Am J Pathol 1999; 155: 1459-66.
16. Rohn TT, Head E, Nesse WH, Cotman CW, Cribbs DH. Activation of caspase-8 in the Alzheimer's disease brain. Neurobiol Dis 2001; 8: 1006-16.
17. Coutinho-Camillo CM, Lourenço SV, Nishimoto IN, Kowalski LP, Soares FA. Caspase expression in oral squamous cell carcinoma. Head Neck 2011; 33: 1191-8.
18. Yu SW, Wang H, Poitras M, et al. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 2002; 297: 259-63.
19. Andrabi SA, Kim NS, Yu SW, et al. Poly(ADP-ribose) (PAR) polymer is a death signal. Proc Natl Acad Sci USA 2006; 103: 18308-13.
20. Yu SW, Andrabi SA, Wang H, et al. Apoptosis-inducing factor mediates poly (ADP-ribose) (PAR) polymer-induced cell death. Proc Natl Acad Sci USA 2006; 103: 18314-9.
21. Hassa PO, Hottiger MO. The diverse biological roles of mammalian PARPs, a small but powerful family of poly-ADP-ribose polymerases. Front Biosci 2008; 13: 3046-82.
22. Hassa PO, Haenni SS, Elser M, Hottiger MO. Nuclear ADP-ribosylation reaction in mammalian cells: where are we today and where are we going? Microbiol Mol Biol Rev 2006; 70: 789-829.
23. Nagasaki K, Manabe T, Hanzawa H, Maass N, Tsukada T, Yamaguchi K. Identification of a novel gene, LDOC1, down-regulated in cancer cell lines. Cancer Lett 1999; 140: 227-34.
24. Nagasaki K, Schem C, von Kaisenberg C, et al. Leucine-zipper protein, LDOC1, inhibits NF-kappaB activation and sensitizes pancreatic cancer cells to apoptosis. Int J Cancer 2003; 105: 454-8.
25. Sharp AJ, Locke DP, McGrath SD, et al. Segmental duplications and copy number variation in the human genome. Am J Hum Genet 2005; 77: 78-88.
26. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 4: 402-8
27. Albrecht S, Bourdeau M, Bennett D, Mufson EJ, Bhattacharjee M, LeBlanc AC. Activation of caspase-6 in aging and mild cognitive impairment. Am J Pathol 2007; 170: 1200-9.
28. Rohn TT, Rissman RA, Davis MC, Kim YE, Cotman CW, Head E. Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. Neurobiol Dis 2002; 11: 341-54.
29. Barnes NY, Li L, Yoshikawa K, Schwartz LM, Oppenheim RW, Milligan CE. Increased production of amyloid precursor protein provides a substrate for caspase-3 in dying motoneurons. J Neurosci 1998; 18: 5869-80.
30. Inoue M, Takahashi K, Niide O, Shibata M, Fukuzawa M, Ra C. LDOC1, a novel MZF-1-interacting protein, induces apoptosis. FEBS Lett 2005; 579: 604-8.
31. Lynn KS, Li LL, Lin YJ, et al. A neural network model for constructing endophenotypes of common complex diseases: an application to male young-onset hypertension microarray data. Bioinformatics 2009; 25: 981-8.
32. Mizutani K, Koike D, Suetsugu S, Takenawa T. WAVE3 functions as a negative regulator of LDOC1. J Biochem 2001; 38: 639-46.