

## REVIEW ARTICLE

---

# Caspase complex in laryngeal squamous cell carcinoma

Aristeidis Chrysovergis<sup>1</sup>, Vasileios S. Papanikolaou<sup>1</sup>, Evangelos Tsiambas<sup>2</sup>, Dimitrios Kikidis<sup>1</sup>, Panagiotis Maragoudakis<sup>3</sup>, Vasileios Ragos<sup>4</sup>, Efthymios Kyrodimos<sup>1</sup>

<sup>1</sup>1<sup>st</sup> ENT Dept, Hippocraton Hospital, University of Athens, Athens, Greece; <sup>2</sup>Dept of Pathology-Cytology, 401 GAH, Athens, Greece; <sup>3</sup>2<sup>nd</sup> Otolaryngology Dept, Attikon University Hospital, Athens, Greece; <sup>4</sup>Dept of Maxillofacial Surgery, Dept of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece.

## Summary

Caspases (cysteine-aspartic proteases) represent a family of enzymes that modify several functions crucial for cell homeostasis such as inflammation and apoptosis. According to their implication in the apoptotic pathways, caspases are characterized as initiators and executioners, respectively. In the first group have been inserted caspase-2,-8,-9, and -10, whereas caspase-3,-6, and-7 belong to the second category. All of these normal actions of the caspase complex that induce apoptosis are altered in carcinoma progression and establishment. In cancer tissues, programmed cell death is inhibited due to a deregulation in expression of apo- and anti-apoptotic proteins. This genetic imbalance drives the cancer cell to immortalization which reflects the aberrant tissue proliferation. For this reason, caspases and the other

apoptotic molecules are considered as important targets for specific targeted therapeutic strategies for enhancing the apoptotic levels inside the malignant tumor cells cores. In the current review we explored the role of caspases deregulation in laryngeal squamous cell carcinoma (LSCC). In malignancies -including LSCC- its deregulation leads the cells to immortalization due to apoptosis inhibition and telomerase overexpression. Caspase-dependent apoptotic rates are decreased in LSCC. For this reason, caspases are considered a very promising target for applying targeted therapeutic strategies in order to enhance apoptosis in the corresponding patients suffering of LSCC.

**Key words:** apoptosis, carcinoma, caspase, larynx,

## Introduction

Tissues homeostasis is critical for the corresponding functions provided by their activity. Imbalances in genes that normally control cell survival and death lead to an extensive, abnormal cell life. Cell immortalization, also due to telomerase aberrant expression, is a frequent genetic event during the multi-step carcinogenetic process [1]. In order to prevent this malfunction, a programmed form of death has been developed inside every cell involving cytoplasmic and nuclear protein cascades in different but interacted pathways [2]. Apoptosis is the term that refers to the genetically programmed cell death mediated by a complex of proteins which influence positively or negatively intrinsic and

extrinsic pathways. In this energy consumption (ATP-dependent) based procedure, enzymes such as proteases and endonucleases split and break down the main domains of the cell entity: cytoplasm and nucleus. The morphological result of this activity is shrinkage of the cell volume combined with condensation of the cytoplasmic micro-environment. Nuclear pyknosis due to chromatin condensation and also DNA fragmentation follows cytoskeleton disorganization and disruption. Finally, the cell is transformed to apoptotic bodies enclosing cellular components exposed to phagocytosis mediated by macrophages [3]. Two main pathways are involved in the previous described apoptotic procedure:

---

Correspondence to: Evangelos Tsiambas, MD, MSc, PhD.  
17 Patriarchou Grigoriou E´ Street, Ag. Paraskevi, 153 41 Athens, Greece.  
Fax: +30 210 6526259, E-mail: tsiambasecyto@yahoo.gr  
Received: 04/05/2018; Accepted: 29/05/2018

intrinsic and extrinsic, respectively. In both of them, several proteins are characterized as inducers or inhibitors of apoptosis [4]. The first uses mitochondrial proteins with prominent the cytochrome C from the inter-membrane space of the organelle. Its activity in the cytoplasm activates caspases (especially caspase-9) complex under the control of p53 and Bcl-2 (B-cell lymphoma-2) proteins. In this version, the apoptotic signal is triggered by inside the cell stress conditions including hypoxia, DNA damage, and altered protein accumulation. Concerning the extrinsic pathway, this is based on receptor-ligand complexes that are activated when the cell receives on its surface (membrane) the corresponding signals from the intercellular environment. The main receptors and their ligand binding molecules are tumor necrosis factor receptor-1(TNFR-1)/tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and Fas Receptor (FasR)/Fas ligand (FasL). In fact, biochemically apoptosis, as a natural cell death mechanism is characterized by the production and development of an intracellular domain called "apoptosome" [5]. It can be described as a multi-protein complex structure including cytochrome C and Apaf-1 (apoptotic protease activating factor-1) which activates caspases interacting also with Bcl proteins. In the current review we explored the role of caspase complex in laryngeal squamous cell carcinoma (LSCC).

### The caspase protein family: genes and functions

Among the proteins that are involved in apoptosis, caspases are critical molecules acting as its inducers. Caspases (cysteine-aspartic proteases) represent a family of enzymes that influence several functions crucial for cell homeostasis such as inflammation, pyroptosis (a distinct aspect of programmed cell death mediated by microbial infection that triggers also an immune response) necroptosis, tissue differentiation and development in the embryonic early stages of life [6]. They also act as tumor suppressor genes, whereas their role in the ageing process is under investigation. Approximately, 15 proteins' proteases have been identified and cloned, implicating 8 chromosomes (1, 2, 4, 7, 10, 11, 16, and 19). The corresponding protein products are initially inactive (pro-caspases) enzymes. Their dimerization or oligomerisation creates the final functional heterotetramer domain due to a cleavage process which develops an active heterodimer complex consisting of two units: a small and large one. According to their implication in the apoptotic pathways, caspases are characterized as initiators and executioners,

respectively. In the first group caspase-2,-8,-9, and -10 have been inserted, whereas caspase-3,-6, and -7 belong to the second category [7]. Concerning the previous described intrinsic apoptotic pathway, the most important protease is caspase-9 which is recruited and activated by cytochrome C/APAF-1 complex. Caspase-9 triggers the activation of the executioner caspases-3 and -7, leading finally to many proteins cleavage inside the cytoplasm. In contrast to this, another critical protein, caspase-8, is activated in the extrinsic pathway by the FasL binding to FasR leading also to recruitment and overexpression of executioner caspases-3,-6, and -7. The result is the degradation of cellular proteins and organelles, a destruction similar to the previous referred action of caspase-9 [8]. Novel studies have also shown that caspase-8 is implicated in cell adhesion and migration, increasing its role in cell homeostasis and interactions inside the tissues [9]. As it has been mentioned before -besides apoptosis- caspases are involved also in the pyroptosis process. Caspase-1,-4,-5, and-11 are involved in this inflammatory procedure. Especially, caspase-1 activates a variety of pro-inflammatory cytokines and then secretes their mature products, interleukins (IL) IL-1 $\alpha$ , IL-1 $\beta$ , and IL-18. In addition, there exists an initial evidence that the complex caspase-1/caspase-7 activates the transcription of nuclear factor- $\kappa$ B (NF- $\kappa$ B), a molecule that modifies the function of TNF, IL-6, and IL-8 [10]. All of these normal actions of the caspase complex that induce apoptosis are altered in carcinoma progression and establishment. In cancer tissues, programmed cell death is inhibited due to a deregulation in the expression of pro- and anti-apoptotic proteins. This genetic imbalance drives the cancer cell to immortalization which reflects the aberrant tissue proliferation. For this reason, caspases and the other apoptotic molecules are considered as important targets for specific targeted therapeutic strategies for enhancing the apoptotic levels inside the malignant tumor cells cores [11].

### Caspase deregulation in LSCC

LSCC is characterized by an aggressive phenotype including poor prognosis, moderate response rates to chemo-radiotherapy and targeted therapeutic agents, such as monoclonal antibodies (mABs) or tyrosine kinase inhibitors (TKIs). In fact, at the time of diagnosis, 60-70% of the patients present with advanced disease. Interestingly, HPV-associated Head & neck squamous cell carcinoma (HNSCCs) demonstrate differences regarding gender, molecular, epidemiological, and prognostic features compared to alcohol and tobacco depended ones

[12]. Recently published experimental studies have shown that specific drug agents modify caspase activity in LSCC leading to altered apoptotic rates. A study group analyzed the effect of combined or not cisplatin/ cetuximab targeted therapy that induce endoplasmic reticulum (ER) stress associated apoptosis in a subset of LSCC patients with overexpression of thioredoxin domain containing protein 5 (TXNDC5). They reported that inhibition of the TXNDC5 protein leads to increased influence of cisplatin in the apoptotic process by enhancing caspase 3 activity [13]. Similarly, the mAB cetuximab positively regulated ER stress associated apoptosis in LSCC cells by inhibiting the expression of TXNDC5 protein. Another study group explored the role of Beclin1 protein expression in LSCC cell cultures. The authors concluded that the molecule's activity is modified *in vitro* by cisplatin application. In fact, a combination of decreased Bcl-2 and increased caspase-8/-9/-3 expression was identified under the influence of cisplatin treatment leading to elevated apoptotic rates [14]. Besides the previously referred agents, natural flavonoids, such as luteolin, seem to demonstrate anti-cancer activities, including increased apoptotic rates. A study group reported that luteolin affects critically Hep-2 cell cultures by inhibiting cell proliferation and enhancing apoptosis by activating the complex caspase-3/caspase-8 [15]. Concerning caspase-8, its downregulation in HPV-positive human squamous cell carcinomas leads to decreased apoptotic rates affecting negatively cisplatin-based treatment [16]. Another natural agent, the epigallocatechin-3-gallate (EGCG), the most important substance of green tea polyphenol, is considered as a significant cell proliferation inhibitor and also a positive apop-

totic agent by activating caspase-3 combined with decreasing telomerase activity [17]. Interestingly, some drugs with antibiotic activity, such as bleomycin-A2, have demonstrated an anti-tumour role by increasing caspase-dependent apoptotic rates *in vitro* in Hep-2 laryngeal carcinoma cells [18]. In addition, another natural formed agent - galangin - isolated from propolis and *Alpinia officinarum* Hance, acts as a suppressor factor in LSCC cells by inhibiting malignant cell proliferation and inducing caspase-3 expression leading to increased apoptotic rates [19]. Furthermore, novel genetic markers that modify the sensitivity of malignant cells to targeted therapeutic strategies and also to the new immunotherapeutic regimens seem to be closely related to apoptotic pathways. Immune inhibitory receptor programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) complex is a major target for specific immunotherapy via monoclonal antibodies, such as nivolumab. A study group analyzing the PD-1/PD-L1 molecules suggested that the altered genes affect negatively apoptosis via caspase-7 overexpression [20].

In conclusion, the caspase complex is a major regulator in apoptosis. In malignancies -including LSCC- their deregulation leads to cells' immortalization due to apoptosis inhibition combined with telomerase overexpression. Caspase-dependent apoptotic rates are decreased in LSCC. For this reason, caspases are considered a very promising target for applying targeted therapeutic strategies in order to enhance apoptosis in patients suffering of LSCC.

### Conflict of interests

The authors declare no conflict of interests.

### References

1. Chung C. Restoring the switch for cancer cell death: Targeting the apoptosis signaling pathway. *Am J Health Syst Pharm* 2018;75:945-52.
2. Arumugam A, Abdull Razis AF. Apoptosis as a Mechanism of the Cancer Chemopreventive Activity of Glucosinolates: a Review. *Asian Pac J Cancer Prev* 2018;19:1439-48.
3. Pfeffer CM, Singh ATK. Apoptosis: A Target for Anticancer Therapy. *Int J Mol Sci* 2018;19:E448-54.
4. Banoth B, Cassel SL. Mitochondria in innate immune signaling. *Transl Res* 2018; 5244:30115-4.
5. Dorstyn L, Akey CW, Kumar S. New insights into apoptosome structure and function. *Cell Death Differ* 2018;25:1194-1208.
6. Anja P, Anahid J, Janko K. Cysteine cathepsins: Their biological and molecular significance in cancer stem cells. *Semin Cancer Biol* 2018;S1044-579X(18)30028-2.
7. Kopeina GS, Prokhorova EA, Lavrik IN, Zhivotovsky B. Alterations in the nucleocytoplasmic transport in apoptosis: Caspases lead the way. *Cell Prolif* 2018;e12467-77.
8. Wilson CH, Kumar S. Caspases in metabolic disease and their therapeutic potential. *Cell Death Differ* 2018;25:1010-24.
9. Keller N, Ozmadenci D, Ichim G, Stupack D. Caspase-8 function, and phosphorylation, in cell migration. *Semin Cell Dev Biol* 2018;82:105-17.
10. Yuan YY, Xie KX, Wang SL, Yuan LW. Inflammatory

- caspase-related pyroptosis: mechanism, regulation and therapeutic potential for inflammatory bowel disease. *Gastroenterol Rep* 2018;6:167-76.
11. McArthur K, Kile BT. Apoptotic Caspases: Multiple or Mistaken Identities? *Trends Cell Biol* 2018;28:475-93.
  12. Mangano A, Mangano A, Lianos GD, Roukos DH, Boni L, Dionigi G. Head and neck squamous cell carcinoma and human papillomavirus: epidemiology, treatment and future trends. *Future Oncol* 2015;11:889-91.
  13. Peng F, Zhang H, Du Y, Tan P. Cetuximab enhances cisplatin-induced endoplasmic reticulum stress-associated apoptosis in laryngeal squamous cell carcinoma cells by inhibiting expression of TXNDC5. *Mol Med Rep* 2018;17:4767-76.
  14. Yang M, Yang XM, Yin DH et al. Beclin1 enhances cisplatin-induced apoptosis via Bcl-2-modulated autophagy in laryngeal carcinoma cells Hep-2. *Neoplasma* 2018;65:42-8.
  15. Zhang H, Li X, Zhang Y, Luan X. Luteolin induces apoptosis by activating Fas signaling pathway at the receptor level in laryngeal squamous cell line Hep-2 cells. *Eur Arch Otorhinolaryngol* 2014;271:1653-9.
  16. Toyozumi Y, Arima N, Izumaru S et al. Loss of caspase-8 activation pathway is a possible mechanism for CDDP resistance in human laryngeal squamous cell carcinoma HEP-2 cells. *Int J Oncol* 2004;25:721-8.
  17. Wang X, Hao MW, Dong K, Lin F, Ren JH, Zhang HZ. Apoptosis induction effects of EGCG in laryngeal squamous cell carcinoma cells through telomerase repression. *Arch Pharm Res* 2009;32:1263-9.
  18. Brahim S, Bettaieb A, Kenani A. Deglycosylated bleomycin triggers apoptosis in laryngeal carcinoma cells in a caspase and reactive oxygen species independent manner. *J Oral Pathol Med* 2008;37:352-7.
  19. Wang HX, Tang C. Galangin suppresses human laryngeal carcinoma via modulation of caspase-3 and AKT signaling pathways. *Oncol Rep* 2017;38:703-14.
  20. Heineman TE, Widman A, Kuan EC, St John M. The genetic landscape of programmed death ligand-1 (PD-L1) alterations in head and neck cancer. *Laryngoscope Investig Otolaryngol* 2017;2:99-103.