APOPTOSIS IN NORMAL BRONCHIAL RESPIRATORY EPITHELIUM – BETWEEN CERTAINTIES AND UNCERTAINTIES

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Abstract. The respiratory epithelium lines the conducting airways and functions as a selective barrier interposed between external environment and human body. It is exposed to various aggressive factors such as viral and bacterial microorganisms, or cigarette smoke and other inhaled noxious substances. The normal airway epithelium has its own mechanisms that maintain the integrity of the epithelial barrier and it is relatively refractory to a number of apoptotic stimuli. The up to date data about apoptosis in normal airway epithelium are limited, especially regarding the regulatory factors of this process. The current knowledge concerning the airway epithelium apoptosis regulation needs to be further studied by exploring the Bcl-2 superfamily members, Zn, p21, or peroxiredoxine V and pirine.

Apoptosis or „programmed cellular death” is an essential physiological process in the development of multicellular organisms, keeping constant the tissue cell population during life. The term “apoptosis” was used for the first time in literature by Kerr, Wyllie and Currie (Kerr JF et al, 1972).

Apoptosis involves a series of biochemical events accompanied by morphologic modifications among which the most important are changes in the mitochondrial membrane, chromatin condensation and fragmentation of the DNA. The „decision” of apoptosis comes either from the cell itself or from the neighboring cells in the tissue or from the immune cells (Ameisen JC, 2002).

Caspases have a key role in the apoptotic pathway and this is the reason why the classification of programmed cellular death is currently made according to the apoptosis execution modality through these protein-enzymes (Bratton SB et al, 2000). Caspases are cysteine-dependent aspartate-specific proteases and their catalytic activity depends on the cysteine residue which has a highly conserved site called pentapeptidic site QACRG. Fourteen different types of caspases have been described in mammals until now, while caspase-11 and 12 were only identified in rats. Two groups of caspases are described: a group of proapoptotic caspases that initiate apoptosis, including procaspases-2, -8, -9 and -10 and a group of execution caspases, including procaspases-3, -6 and -7 (Denault JB et al, 2010).

Caspases are synthesized as inactive enzymes called pro-caspases, having at their NH2 terminal a pro-domain that is followed by larger or smaller subunits. Pro-caspases are proteolysed between these subunits and the pro-domain is also frequently removed by cleavage and as a consequence these protein enzymes become activated.

As an intrinsic barrier between outer and inner environment, the respiratory epithelium is directly exposed to inhaled pollutants, viruses and allergens which can induce its direct injury or, through an inflammatory process, an indirect injury.

Histological pattern of the upper respiratory airways epithelium (excepting the olfactory mucosa) and of the inferior ones up to the entrance to the pulmonary lobule (intralobular bronchiole) is a pseudostratified columnar ciliated type, with goblet cells, also called respiratory epithelium.

Six different types of cells compose the normal structure of this epithelium: high columnar ciliated cells (the most numerous), goblet cells (normally rated as one goblet cell for 5

ciliated cells), regenerative cells, brush cells type I and II and endocrine-like cells (Ross MH, Pawlina W, 2010). Through the muco-ciliated barrier, these cells ensure the adhesion and removal of foreign particles and of infectious agents from the inhaled air and moreover, they secrete a series of cytokines and other mediators playing an important role in the regulation of the immune processes and bronchodilation.

By comparison with the alveolar epithelium, the knowledge regarding the apoptosis of normal respiratory epithelium is extremely limited, with only few data on the apoptosis of ciliated cells and that of goblet cells and almost none on that of regeneration cells. Among the most important substances inducing the apoptosis of the respiratory cells most reports are referring to inhaled substances such as cigarette smoke, cytokines (such as transforming growth factor-TGF), infectious agents (such as viruses or bacteria), hypoxia and allergens (Gelbman BD et al, 2007; Zalewski PD, Ruffin RE, 2008).

Beside the participation in the occurrence of epithelial lesions and in the inflammation regulation, the apoptosis of the respiratory cells contributes to the maintenance of the normal amount of cells. Also, apoptosis is the mechanism involved in limitation of viral infections or other epithelial lesions (allergen-induced or induced by other irritants) (Zalewski PD, Ruffin RE, 2008; Tesfaigzi Y, 2008). Moreover, the intensification of the apoptosis phenomenon could represent a marker of epithelial lesion (Zalewski PD, Ruffin RE, 2008) in some respiratory diseases.

The mitochondria play a very important role in the normal cell biochemistry. Alongside its role in mediating and amplifying the apoptotic signals, secondary to DNA injury or other aggressive factors such as physical and chemical stress, mitochondria has a key role in generation and dispersion of cell death signals originating within cells (Kroemer G, Reed JC, 2000; Kroemer G et al, 2007).

Most inducing factors produce a disturbance of the internal mitochondrial membrane potential that results in an increased permeability for molecules with a molecular weight of more than 1.5 kDa. Concomitantly with the increase in mitochondrial internal membrane permeability a water mitochondrial influx, followed by secondary swelling and the possible rupture of the external mitochondrial membrane, contributing to the cytoplasmic release of proapoptotic proteins (such as cytochrome c) and of other factors such as: apoptosis induction factor (AIF), endoG endonuclease and Htr/Omi (Li LY et al, 2001; Verhagen AM et al, 2002). An interest ing fact is that the increased nuclear membrane permeability is always followed by a disturbance in the mitochondrial internal membrane potential but this event is not reversibly (disturbed potential is not followed by an increased membrane permeability); moreover the cytosolic release of cytochrome c is also noted in the absence of mitochondrial internal membrane potential modification.

Up to date several mechanisms involved in mitochondrial membrane permeability increase have been proposed, but most researchers agree on the idea of “permeability pores” development thought to be formed by an adenine nucleotide translocator (ANT) and of a voltage-dependent anionic channel as a central component of the transmembranar pore, called VDAC (Kroemer G et al, 2007).

Normally all cells including respiratory cells have a strict mitochondrial regulatory system, performed by a series of molecules belonging to the Bcl-2 family of molecules. Two apoptosis control modalities involving the members of Bcl-2 family are suggested: regulation of the caspases activity (the antiapoptotic pathway) or favoring the preservation of mitochondrial

integrity, hence the inhibition of the proapoptotic mitochondrial proteins cytoplasmic release (Marsden V, 2002).

Recently, a series of proapoptotic factors (Bax or Bak) were included in the Bcl-2 family. The way Bax or Bak modify the mitochondrial integrity is not completely elucidated. The current hypothesis is that polymerized Bax and Bak initiate themselves the formation of a permeation channel in the external mitochondrial membrane either interact with some components of the mitochondrial membrane pore such as VDAC (Marsden V, 2002).

It is also possible that several antiapoptotic members of the Bcl-2 family may act by sequestering the proapoptotic members of the Bcl-2 family. Thus the inhibition of Bax or Bak activation or their polymerization and the development of the mitochondrial proapoptotic events could be achieved.

Moreover, Bcl-2 is considered an inhibitor of Apaf-1/caspase-9- independent, caspase-7 dependent apoptotic pathways (Marsden V, 2002). Furthermore, nowadays it is believed that a still unidentified Apaf-1 homologue directly regulated by Bcl-2/Bcl-XL may exist.

Most studies regarding the respiratory pathology demonstrated an increase of Bcl-2 expression in respiratory epithelium cells under exposure to ozone, endotoxins, cigarette smoke or allergens inducing metaplasia. All researchers conclusions support the idea that Bcl-2 expression regulation may lead to a decrease in goblet cells hyperplasia (Tesfaigzi Y et al, 2004; Tesfaigzi Y, 2002; Harris JF et al, 2005).

Nevertheless, limited data referring to Bcl-2 as well as to other members of the Bcl-2 family are currently available. Knowledge of the proteins involvement in the protection of the normal respiratory epithelium and in the development of inflammation may develop new regulatory methods of the cellular death in the respiratory epithelium.

An interesting discovery using immunohistochemical studies was made regarding the evident distribution of procaspase-3 in the apical pole of respiratory epithelium cells. If procaspase-3 was proved to be apically localized consequently its regulatory factors (including apoptosis regulatory factors) should be also placed in the neighboring cellular area. These aspects were certified by results of several studies which proved that Cu/Zn superoxide dismutase (with regulatory role in the apoptotic process) is also disposed in the cellular apical pole (Carter JE et al, 2002), and moreover by the intense immunolabeling for inhibitors of apoptosis (IAPs) in the normal bronchial epithelium (Viscioni B et al, 2006).

This unique spatial arrangement of antiapoptotic and propaoptotic factors in the respiratory epithelial cells involves some sequestering mechanisms. An apical cellular cytoskeleton organized as a dense network of cytokeratin filaments and microtubules may be most probably involved in these mechanisms.

Other partially known regulatory factors could be involved beside Bcl-2 family in the normal respiratory epithelium in the apoptosis cascade. Among these Fas, FasL, Zn, p21, stress-response proteins, peroxiredoxin V, pirine and corticosteroids, may be included. Unfortunately, the existent information is poor and incomplete to facilitate the understanding of the regulatory pathways of this mechanism in the respiratory epithelium and of the rather refractory characteristics to different apoptotic stimuli of this epithelium despite multiple aggressive factors.

The Fas important role in the apoptosis initiation in the hematopoietic line is today unanimously recognized. The expression and role of this molecule in the epithelial tissue is still incompletely elucidated. Recently, research results demonstrated the coexpression of Fas/FasL in normal human bronchial epithelium. Fas and FasL are both expressed on basal, columnar ciliated, and goblet cells surfaces. This coexpression of receptor and ligand is rarely observed in

epithelial cells in human body (Zalewski PD, Ruffin RE, 2008; Druilhe A et al, 1998). The role of Fas/FasL is the initiation of apoptosis or the modulation of the cell turnover rate in the respiratory epithelium. However, cellular turnover rate in normal respiratory epithelium is estimated to be 1%, suggesting the intervention of some regulation modalities for Fas/FasL interaction to prevent apoptosis. The regulation modalities may be represented by the separation of Fas receptor from FasL in nonadjacent membrane surfaces, by phosphorylation of Fas receptor, by metalloproteinase involvement in soluble Fas or cell membrane fixed Fas occurence.

A special interest is nowadays directed toward separation Fas and FasL roles in the bronchial epithelium. Although inconclusive data are available Fas expression seems to be important in the injured areas of the respiratory epithelium. Nevertheless FasL expression in bronchial epithelial cells may prevent local infiltration with inflammatory cells expressing Fas (such as eosinophils).

Thus, FasL expression in bronchial epithelium represents a normal cellular status, while epithelial infiltration by inflammatory cells can be attributed, at least partially, to the inactivation of Fas/FasL barrier. This collapse may be a consequence of the alteration of FasL expression or of a genetic variant of FasL expression exhibiting a decreased protection capacity of the bronchial epithelium against inflammatory processes. This aspect could be considered as a genetic predisposition of respiratory epithelium to chronic inflammatory processes (Gochuico BR et al, 1998).

Zn (zinc) can be included among factors contributing to the normal respiratory epithelial cells resilience against apoptosis. In an experimental model, Zn reduced the accumulation of eosinophils in the lamina propria in induced respiratory mucosal inflammation (Lang CJ et al, 2007; Richter M et al, 2003). More than a caspase inhibitor, Zn is also an antioxidant and a membranar stabilization factor (Truong-Tran AQ et al, 2003). Moreover, different authors suggest that the administration of Zn supplements may represent a protection factor against respiratory epithelial cells lesions (Bao S, Knoell DL, 2006). This fact is also supported by decreased Zn concentrations in some chronic inflammatory respiratory illnesses (Zalewski, P. D, 2006).

Another control factor of respiratory epithelial cells apoptosis is p21 or Cip1/WAF1, a cyclin-kinase dependent inhibitor involved in cell proliferation and death regulatory mechanism (Maddika S et al, 2007). Experimental studies performed on genetically modified rats deprived of p21 gene expression demonstrated an increased apoptosis in respiratory epithelial cells compared to normal rats (Blundell R et al, 2004). Supplementary, some researches suggested that p21 could be one of the factors involved in normal turnover of respiratory epithelial cells in chronic inflammatory diseases (such as asthma). p21 expression regulation may be one of the new therapeutic targets mainly as its expression proved to be irresponsive to corticosteroid therapy (Puddicombe SM et al, 2003).

Stress response proteins (such as Hsp-70 shock protein) ensure cells protection against several pathogens. The involvement of these stress-response proteins in the protection of respiratory epithelial cells against proteases release from granulations of neutrophils was quite recently demonstrated (Ito H et al, 2006). Nevertheless, further investigations regarding the role of these proteins in respiratory epithelium are necessary.

Understanding the exact mechanisms of cigarette smoke involvement in bronchial epithelial cells lesions represents a real interest for the researchers, especially finding new modalities to modulate its regulating factors. It was experimentally proved that normal respiratory epithelial cells express important quantities of peroxiredoxine V (PRXV), an

antioxidant protein (Serikov VB et al, 2006). Moreover, other studies proved that pirine could be an intensely expressed protein in human bronchial epithelial cells exposed to substances from cigarette smoke and associated with an important increased cell apoptosis (Gelbman BD et al, 2007). However, the literature data regarding peroxiredoxine V and pirine involvement in bronchial epithelium apoptosis is extremely scarce, so further studies are waited to support current findings.

In the category of apoptotic factors taken nowadays into account in the respiratory epithelium, some drugs, currently used in respiratory pathology, especially in chronic inflammatory diseases requiring the administration of corticosteroids, can be included (Dorscheid DR et al, 2003; White SR, Dorscheid DR, 2002). According to the results of some experimental researches, corticosteroids lead to a perturbation in mitochondrial polarity, to the caspases activation and apoptosis induction in bronchial epithelium, in cell cultures (Dorscheid DR et al, 2006).

Thus, there are numerous researchers which consider that an apoptosis increase noted in some respiratory diseases can be partially attributed to the administration of corticosteroids and not only to the disease itself.

Apoptosis is a normal process unrecognized as a distinct mechanism of cellular death until the second half of the past century. This achievement was logically followed by the evaluation of the importance of this process both in normal tissue physiology and in the physiopathology of some illnesses.

Research and correspondent obtained information of normal apoptosis in bronchial respiratory epithelium is quite recent, being obtained by the wide application of fibrobronchoscopy associated with bronchial biopsy as an investigation method in respiratory pathology. Current data are incomplete especially regarding oxidative stress and hence epithelial repair phenomenon with a special reference to the biology of local growth factors.

Eventually, cellular and molecular biology data regarding respiratory epithelial cells need further researches. Certainly, respiratory epithelial cells communicate and a single cell’s activity can influence the neighboring cells. Thus the bidirectional intercellular physical and biochemical interaction may regulate ciliated and goblet cells death.

All this information will unquestionably contribute to a better understanding of physiopathology notions on respiratory illnesses involving the mucosa and the respiratory epithelium, developing new premises of a precise target of the therapeutic approach, decreasing side effects number and severity, and improving the patient’s quality of life.

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