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Chronic Obstructive Pulmonary Disease – Chaperonopathology

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1. Introduction

Cigarette smoking is clearly associated with the development of chronic airway obstruction pulmonary disease and is responsible for 80–90% of cases. However, only 15–20% of heavy smokers develop clinically significant airflow obstruction. In the rest pulmonary function remains within normal limits. Besides the risk factors that are involved in airway obstruction, the genetic predisposition is also considered a key factor. It modulates lung's response to cigarette smoke inhalation and the development of airway obstruction. In addition to smoke induced emphysema, genetic susceptibility leading to α1-antitrypsin deficiency is associated with the propensity for the development of early-onset, familial emphysema. Thus both environmental and genetic factors contribute to the pathogenesis of emphysema.

The molecular basis for tobacco smoke-induced emphysema is poorly understood. To thoroughly unravel the cellular and molecular events or signaling pathways that may contribute to the pathogenesis of smoke-induced emphysema or COPD, gene expression profiling - serial analysis of gene expression (SAGE) and microarray analysis as well as proteomics have been recently applied. The gene expression profiles of lung tissues from control smokers (GOLD-0) and moderate (GOLD-2) COPD smokers identified numerous classes of genes, the expression of which is altered in COPD patients. These include genes encoding molecules for signal transduction, receptor function, growth factor, nuclear chromatin and DNA binding, adhesion and cytoskeleton, metabolism, matrix, cell cycle, and oxidative stress such as HSP70 protein, heme oxygenase (decycling) 1 (HO-1). The data from proteomics also confirms a large number of proteins related to cigarette smoke induced endoplasmic reticulum stress, repair/injury proteins, heat shock proteins, apoptosis and cell cycle responsible molecules.

COPD is obviously a disease of imbalance of proteins - oxi/antioxidant, protease/antiprotease, apoptosis/proliferation, acetylases/deacetylases, that can no longer perform their proper function to keep the homeostasis in the new environmental settings of the oxidative stress.

Molecular chaperones provide the functional activity of proteins, they counteract the formation of abberantly folded polypeptides and allow their correct refolding under stress

recovery. Chaperones are responsible for protein folding and allow the functional state of cells to be maintained, by preventing irreversible protein unfolding and aggregation. Numerous studies, over the last decade have investigated the structural and functional characteristics of molecular chaperones, classifying them in families based on size, structure and activity. Today there are more than 25 families of molecular chaperones, with more than 100 proteins participating in the folding events in the mammal. These include a group of proteins, known as heat shock or cell stress proteins.

Heat shock or cell stress response is one of the most evolutionary conserved protective mechanisms in cells. It is stimulated under ''stress'' (thermal, metabolic, oxidative, etc), when the conditions of the cell environment are deleterious and alter the protein folding and their proper biological activity. Cell stress involves the temporary modification of gene expression and synthesis of different heat shock protein family members. They help the cell and the organism to cope with environmental or physiological stress. Some of the heat shock proteins are constitutively expressed in non-stressed conditions and act as intracellular chaperones towards fundamental cellular processes – cytoskeletal architecture, mutation masking, protein transport, translation regulation, intracellular redox homeostasis, protection against spontaneous or stimulated programmed cell death - apoptosis. Others are synthetised in response to stress to prevent protein aggregation, refold damaged proteins. Heat shock proteins could also participate in the protein triage and modulate the ubiquitin – proteasome pathway, promoting the degradation of irreversibly denatured proteins.

While the cellular protein management of heat shock response and stress proteins is well described their role in the immune/inflammatory responses in multicellular organism is still elusive. As an anti-inflammatory effector the heat shock proteins influence cytokine signal transduction and gene expression by inhibiting the translocation of the transcription nuclear factor kappa B (NF-kB) to the nucleus. In this aspect they regulate the synthesis of inflammatory mediators. As proinflammatory mediators, necrotic and non-necrotic release of constitutively expressed or stress induced heat shock proteins, into the extracellular environment, produce a multifaceted immune/inflammatory response. They activate immune effector cells and stimulate cytokine release. Therefore the ability of heat stress response to modulate inflammation is an important aspect of a variety of pathophsysiological states, characterized by dysregulated inflammatory response.

Cell stress proteins are reported to be positively correlated to longevity and capacity for mounting a cell stress response, implying the fact that chaperones are essential adaptive mechanisms for survival. Unfortunately chaperone levels generally decrease or become functionally incompetent with age. The accumulation of misfolded proteins that occurs with senescence results in a chaperone deficiency and leads to the onset of degenerative or age related diseases. A shift in the balance between misfolded proteins and available free chaperones in ageing organisms can bring about defects in signal transduction, protein transport, cellular organization and immune functions.

Age-related post-translation modifications of proteins can seriously curtail or change their functions and thus give rise to proteinopathies of ageing, a hallmark of senescence at molecular level. A normal set of chaperones would potentially prevent the deleterious effect of proteinopathies. However chaperones also are being modified with the passage of time. These acquired chaperonopathies are likely to contribute significantly to senescence and lower the quality of life in the elderly.

In conclusion normal chaperones, particularly cell stress proteins are important in cell physiology at all ages. They are responsible for protein folding, functioning and homeostasis and play critical roles as major cellular anti-stress and anti-disease mechanism. Defective chaperones are most probably an additional factor, accompanying the development and progression of senescence and age-associated diseases (neurodegenerative, cancer, atherosclerosis, COPD) most of which are aggrevated by stress.

In the current chapter we shall represent COPD as a chaperonopathy (proteinopathy). We shall concentrate on the current data from research studies, concerning the molecular pathology of COPD, studies that are shedding light on the participation of stress molecules in COPD initiation and progression. We shall also comment the relation of chaperones to already known pathological mechanisms, their clinical application as diagnostic markers for COPD, as well as markers for NSCLC early detection.

2. Cell stress or heat shock proteins

All organisms respond to potentially harmful environmental factors by an up-regulation of heat shock protein expression. Cell stress or heat shock proteins were first discovered in 1962 by Ritossa who observed a pattern of Drosophila salivary gland chromosome puffs induced under transient exposure to high temperature. It was subsequently described that these highly conserved group of proteins could be induced by many other stress factors. Mammalian heat shock proteins are classified into two groups according to their size: high molecular weight heat shock proteins (HSP) and small HSP - sHSP. The first group includes three major families: HSP90, HSP70 and HSP60. Some of these proteins are constitutively expressed (in-house chaperones), whereas the expression of the others is induced by stressful conditions. High molecular weight stress protein are ATP-dependent chaperones and require co-chaperones to perform their ATP-binding and modulate their conformation. In contrast sHSP are ATP-independent. Heat shock proteins are expressed in both normal and stress conditions and are responsible for:

1) facilitating the proper folding of nascent proteins in cytosol, endoplasmatic reticulum, mitochondria; 2) import of proteins into cellular transport; 3) prevention of protein aggregates, refolding of denatured proteins; 4) degradation of unstable proteins; 5) control of apoptosis.

HSP also participates in the intracellular transport and have been implicated in the loading of immunogenic peptides in histocompatibility complexes (MHC) in the T-cell presentation.

2.1 High molecular weight heat shock proteins

The HSP70 family is the most highly conserved and best studied class of HSP. Human cells contain several HSP 70 family members – constitutively expressed, inducible, mitochondrial – HSP75, and GRP78, localized in the endoplasmatic reticulum. Under normal conditions HSP70 proteins function as ATP-dependent chaperones, assisting the folding of newly-synthesised proteins, participating in intracellular transport of proteins across cellular membranes. Under stressful conditions the synthesis of inducible HSP70 enhances the ability of cells to cope with the increased levels of denatured proteins. HSP70 blocks caspase-depedent and independent activation of apoptosis (Shi et al,1992; Murakami et al,

1988;) It participates in the ubiquitination of proteins through its co-chaperones – BAG1 and CHIP. (Meacham et al, 2001; Luders et al, 2000)

The HSP90 family include ATP – dependent chaperones – HSP90α and HSP90β and GRP94. The two isoforms of HSP90 (HSP90α and HSP90β) that are essential to cells are abundantly expressed under normal conditions (Csermely et al, 1998). HSP90 proteins make up 1-2% of the cytosolic proteins and are additionally synthethised during stress. They participate in cell signalling pathways –ligand dependent transcription factors – Glucocorticoid receptor (Nathan et al, 1995); ligand independent transcription factors – Myo-D, tyrosine and serine/threonine kinases (Hartson et al, 1994; Shaknovich et al, 1992). Their chaperone function is almost entirely limited to these transcription factors and signal transducing kinases. HSP90 family members also have anti-apoptotic functions and stimulate the protein triage (Tsubuki et al, 1994; Lewis et al, 2000).

HSP60 is called chaperonin. It is constitutively expressed, found primarily in the mitochondrial matrix, although up to 15% could be cytoplasmically expressed. It is ATP-dependent chaperone, protecting the mitochondrial proteins and facilitating the proteolytic degradation of misfolded proteins. The chaperone function of HSP60 is regulated by a co-chaperone, known as HSP10 that modulates substrate binding and ATP-ase activity. In the presence of ADP, HSP60 regulates apoptosis, demonstrating both pro- and antiapoptotic functions (Bukau et al, 1998).

2.2 Small heat shock proteins

The small heat shock proteins constitute of a diverse family of ubiquituous intracellular proteins (Arrigo et al, 1998). In human ten different sHSP have been described but only a few of them (HSP27, HSP22 and α-Bcrystallin (HSPB5) are true heat shock proteins expressed in response to stress. sHSP are characterized by small molecular weight (12-43kDa) and a conserved C-terminal domain (the α-crystallin domain). They share the ability to form globular oligomeric structures with molecular masses ranging between 50-800kDa. The dynamic organization of these proteins is essential for performing their biological activity. It depends on their phosphorylated status which is performed by specific signal transduction pathways. It is generally assumed that stress favors the formation of large oligomers associated with unfolded proteins while phosphorylation does the reverse. Large unphosphorylated oligomers of sHSP have greater potentiality to protect cells through their ability to perform chaperone activity. The formation of small phosphorylated oligomers may be required for the binding of unfolded proteins as well as for the recycling of the larger ones (Kato et al, 1996).

2.2.1 sHSP5

α-crystallin, a major structural protein of the vertebrate eye lens, is the most intensively studied representarive member of sHSP family. α-crystallin is one of the three major crystallins of the vertebrate eye lens (Ingolia et al, 1982). However it became a major focus of studies since 1982 when Drosophila sHSP were found to share sequence similarities with α-crystallin. Soon after, it was shown that α-crystallin has other functions, defining it as a molecular chaperone – prevents the thermal aggregation of various proteins, including the lens proteins. In the lens α-crystallin exists as a heteropolymer with the

molecular size of approximately 800kDa, having up to 40 subunits from two gene products – αA and αB. αA is encoded by and constitutes of 173 aminoacids, while αB is encoded by and has 175 aminoacids and both share 57% sequence similarity. In contrast to αA, αB is also constitutively expressed in various tissues with high rates of oxidative stress – skeletal muscles, brain, heart, kidney (Lowe, 1992). Its primary sequence can be organized in three distinct structural regions: an α-crystallin domain of 90 amino-acids in length which is conserved among all sHSP and flanked by an N- and C – terminal domains of variable length and sequence. The conserved α-crystallin domain spans residues 68-148. It is has seven strands, organized in two sheets. The N - terminal domain is highly variable and influences subunit oligomerization and chaperone-like activity, whereas the C-terminal extension stabilizes the global structure and enhances protein/substrate complex (Sun et al, 1997; Bhatacharyya et al, 2002) αB-crystallin is a major structural protein of human lenses that belongs to the family of small heat-shock proteins. It has auto-kinase activity and participates in intracellular architecture and membrane stabilisation (Nicole et al, 2002; Wang K, Spector A, 1996). It acts as molecular chaperone and stabilises proteins in large soluble aggregates in the cytoplasm. The cytoplasmic expression of αB-crystallin is also responsible for the regulation of cyclin-D1 ubiquitination (Liu et al, 2006) and inhibition of pro-apoptotic proteins such as caspase-3, p53, Bax and Bclxs (Mao et al, 2004; Lin et al, 2007)

3. Oxidative stress, COPD and heat shock proteins

Compared to other organs lungs are unique in their exposure to high levels of oxygen. Because of their close contact with the environment the airway epithelium is directly exposed to either exogenous oxidants – (cigarette smoke, airway pollutants), or endogenous ones – generated by phagocytes or other cell types. To keep the balance lungs need efficient adaptive mechanisms that correspond to their physiological functions. If the enzymatic or non-enzymatic antioxidant systems do not provide the corresponding adaptive response oxidative stress occurs.

It is still considered that oxidative stress is one of the triggers, contributing to the enhanced or abnormal inflammatory response, characteristic for COPD patients.

3.1 Oxidative stress, chaperones and epithelial injury in COPD

The airspace epithelial surface is particularly vulnerable to the effects of oxidative stress. The injury of the epithelium is an important early event, following exposure to cigarette smoke. The noxious effects of the cigarette smoke on human epithelial cell monolayers has been demonstrated by cell detachment, decreased cell adherence and increased cell lysis (Jones, et al, 1980; Lannan S t al, 1994). It is supposed that these effects are in part oxidant mediated since GSH appears to be critical for the maintenance of the epithelial integrity following exposure to smoke. It is demonstrated in studies that the direct exposure to smoke condensates is associated with profound changes in the homeostais of glutathione (GSH) (Li et al, 1994, 1996). Concentration of GSH are significantly decreased after exposure to cigarette smoke condensate. This is due to a decrease of the activity of the enzymes, responsible for the keeping the redox-cycle – glutathioneperoxidase, glucose-6-phosphate dehydrogenase. In addition the depletion of GSH alone induces airway detachment and increases its permeability (Li et al, 1995; Rahman et al, 1995).

The small heat shock proteins – HSP27 and αB crystalline have antioxidant ability and increase cell resistance to oxidative injuries (Arrigo et al, 2001). It is reported (Yan et al, 2002) both in cell cultures and whole animals that their expression correlates to decreased levels of reactive oxygen species (ROS) and nitric oxide (NO•).(Preville et al, 1999; Mehlen et al, 1996) Consequently, in cells exposed to oxidative stress, sHSPs lower the levels of lipid peroxidation (Fridaus et al, 2006; Preville et al, 1998). They maintain the mitochondrial potential and provide the production of ATP, thus corresponding to both increased energy needs for stressed cells on the one hand, and ATP supply for the functional activity of the other chaperones on the other (Paul et al, 2000).

The antioxidant activity of HSP27 and αB crystalline is performed by the increase of the levels of gluthatione. (Mehlen et al, 1996) They induce the up-regulation of glucose-6-phosphate dehydrogenase – the enzyme that provides the reducing power of the cell, by reducing NADP+ to NADPH(H) + . In addition it is recently observed that HSP27 and αB-crystalline expression decreases iron intracellular levels. Thus they prevent the Fenton reaction and the formation of hydroxyl radical (OH•) (Arrigo et al, 2005; Chen et al, 2006).

Ruicheng Hu et al, 2011 performed proteomic analysis and found that the expression of HSP27 was upregulated in smokers, and this upregulation was particularly marked in COPD smokers. The expression of HSP27 between the groups was confirmed by IHC and Western blotting. Based on their results and other studies that have shown a protective role for HSP27 against oxidative stress and apoptosis, it could be suggested that induction of HSP27 protects the lung cells of smokers and COPD patients against oxidative stress and apoptosis.Their   experiments showed that expression of HSP27 was upregulated in the lungs of smokers, and especially smokers with COPD, even though there was no difference in smoking index between smokers with or without COPD. Therefore, it could be suggested that the upregulation of HSP27 expression in smokers is primarily due to oxidative stress and partly due to inflammation, whereas the difference in HSP27 expression between smokers with or without COPD may predominantly be due to inflammation. HSP27 is a multi-functional cytoprotective factor that protects cells from oxidative stress by regulating the activity of several detoxifying enzymes and promoting the degradation of misfolded proteins. HSP27 also protects cells from unfavourable stimuli by playing a role in apoptosis/survival signal transduction pathways (Ito et al, 2003; Bruey et al, 2000). Phosphorylated HSP27 is a ubiquitin-binding protein that binds to 16 polyubiquitin chains and thereby enhances the degradation of ubiquitinated proteins by the 26S proteasome (Jackson et al, 2008) By enhancing the degradation of IκB-α and activating the nuclear factor-κB signal transduction pathway, HSP27 promotes cell survival under conditions of stress. (Yu et al, 2008; Kuoyt et al, 1995)

Another function of the sHSPs that triggers the interest towards their participation in the lung epithelial injury is that both of them are responsible for the protection of cytoskeleton.((Benndorf et al, 1994; Mounier et al, 2002) Small HSPs are involved in the control of cytoskeletal organization during heat and oxidative stress. They maintain the polymerization-depolymerization processes of F-actin and thus are directly responsible for both cell integrity and intracellular contacts. (Jog et al, 2007; Singh et al, 2007; Mairesse et al, 1996). In addition αB-crystalline is a well-known stabilizer of the intermediate filaments and play a major role in cytoskeletal architecture homeostasis (Bennardini et al, 1992). It is demonstrated in epithelial cells that cell signalling pathways, activated by disrupture of

both microfilametns, intermediate filaments and microtubules, lead to the phosphorylation of αB-crystalline, underlying the biological importance of this heat shock protein in preserving the integral cell architecture (Launay et al, 2006).

Cherneva et al, studied the tissue expression of αB crystalline in 28 COPD patients, 14 with age-related emphysema and 23 smokers without COPD. Immunohistochemistry towards αB crystalline was applied. Results were evaluated semiquantitaively. No nuclear staining was present. Only cytoplasmic staining was observed. In most of the cases there was a homogeneous staining among cells. Two patients with COPD had moderate; 26 had intensive staining. In age-related emphysema 5 patients had weak; 2 had moderate and 7 had intensive staining. In smokers without COPD no staining was detected. The clinical implication of our preliminary results needs further investigation (Fig.1).
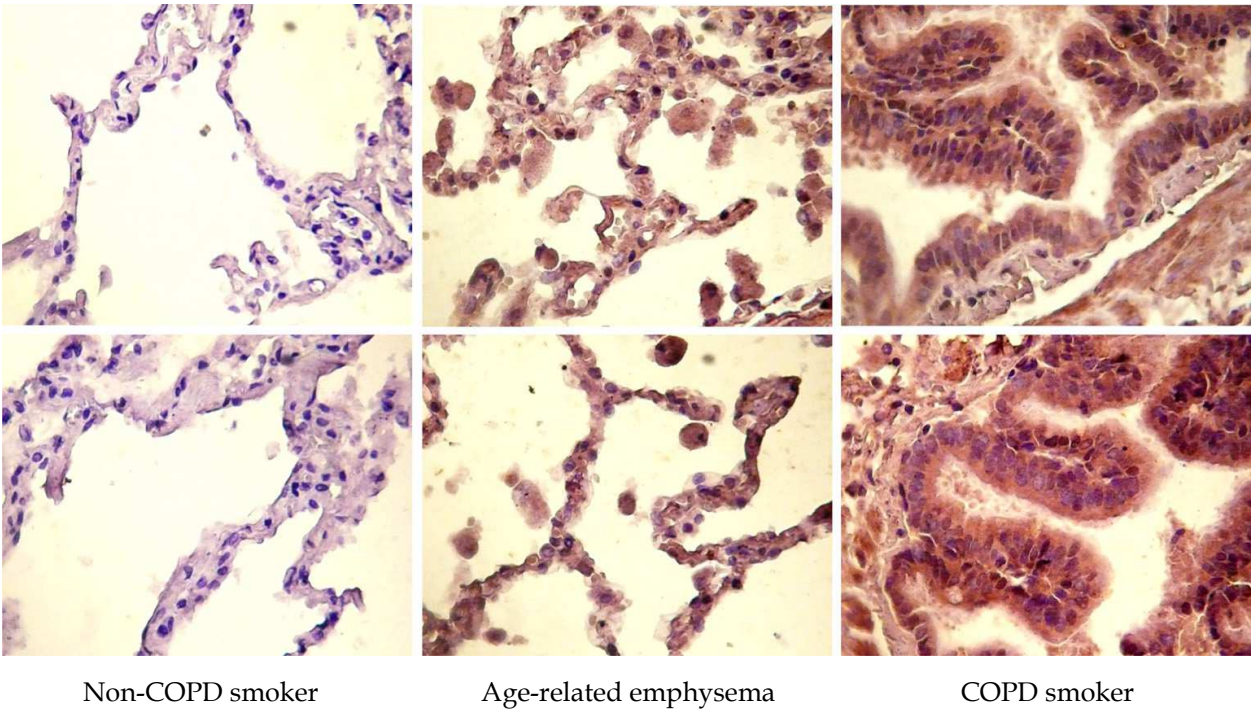

| Non-COPD smoker | Age-related emphysema | COPD smoker |

Fig. 1. Tissue expression of αB-crystalline in lung tissues from non-COPD smokers, age-related emphysema and COPD patients.

The levels of αB-crystalline were measured in the fixed lung sections (3-mm thick) by immunohistochemical staining using rabbit polyclonal anti-αB-crystalline antibody (1:500 dilution) with avidin–biotin–peroxidase complex method followed by hematoxylin counter staining. Brown colour and the variability of its intensity represents the presence of αB-crystalline.The assessment of immunostaining intensity was performed semiquantitatively and in a blinded fashion.

3.2 Oxidative stress, chaperones and neutrophil sequestration in COPD

The oxidant burden in the lungs of smokers can further be augmented by the increased numbers of macrophages (two to four folds) and neutrophils (10-fold). Bronchial biopsies and lung resection studies represent a large number of neutrophils in the lungs and alveolar walls of COPD smokers (Kilburn et al, 1975; Hunninghake et al, 1983).

Neutrophils are first recruited and then sequestered due to size difference between neutrophils and pulmonary capillary vessels (MacNee, 1993). Radiolabelled studies have shown that lungs normally contain a large number of non-circulating neutrophils, which are retained or moving slowly across lung capillary bed (Selby et al, 1991). In comparison to erythrocytes, neutrophils are usually retained and the number of retained cells correlates to their capacity to adapt to the physilologically narrower diameter of the lung capillaries. The less deformable the cells the greater the sequestration of these cells in the pulmonary circulation. The deposition of the cells in microcirculation allows them to interact longer with the endothelium, to adhere to endothelial cells, or transmigrate in the interstitium and alveolar airspaces and respond to the inflammatory cytokines or infections. Thus any conditions that make the neutrophils less deformable create a predisposition to their sequestration in the lung capillary bed.

Studies in humans show that neutrophils and red blood cells are transiently sequesterd in lung capillary bed during smoking and return to the general circulation after smoking cessation. In vitro experiments show that cells exposed to smoke are less flexible. A similar result can be demonstrated in vivo in patients that are actively smoking – their cells lose flexibility. Decreased neutrophil deformability occurs owing to the assembly of the cytoskeleton – polymerization of microfilaments (F-actin), resulting in cell stiffening. It has been suggested that since each puff of cigarette smoke contains $> 10^{16}$ oxidant molecules the effect of cigarette smoking is probably oxidantly mediated. This is confirmed by the fact that the decrease of neutrophil deformability is accompanied by the depletion of GSH (Drost et al, 1992). In addition oxidants affect it by altering cellular cytoskeleton through the polymerization of actin.

The control of F-actin cytoskeleton is mediated by the oligomers of HSP27. It is responsible not only for chemotaxis and cytoskeleton reorganization during migration, but also for processes engaged in exocytosis. It is recently observed that this small heat shock protein regulates neutrophil chemotaxis and exocytosis through the control of actin reorganization. (Jog et al, 2007; Singh et al, 2007)

The role of small heat shock proteins in the maintenance of neutrophil cytoskeleton and their reorganization under oxidative stress and inflammation is not investigated in COPD patients. It could however be speculated that the biological role of this chaperones related to the assembly and dynamics of the cell architecture makes them a target for future research. Since the deposition of neutrophils is triggered by oxidative stress it would be curious to compare the levels of expression of these small heat shock molecules in both COPD smokers, COPD non-smokers and healthy smokers.

3.3 Oxidative stress, chaperones and apoptosis in COPD

It has been proposed recently that COPD is associated with the loss of alveolar endothelial cells as well as lung epithelial cells and that apoptosis can be an essential element of emphysema. Apoptosis in emphysematous lungs is more commonly observed than in nonsmoker's lungs. Peripheral blood leukocytes and lymphocytes of patients with COPD also show increased rates of apoptosis (Tuder et al, 2003; Kasahara et al, 2001). Studies have reported that cigarette smoke induces apoptosis of lung structural cells by oxidative/endoplasmic reticulum stress.When the lungs are insulted with oxidants, consumption of intracellular reductants is increased resulting in aberrant protein folding.

As intracellular chaperones heat shock proteins have anti-apoptotic properties. Gal et al, 2011 showed that cigarette smoke extract stimulates the expression of HSP72 in alveolar epithelial cells, diminishing apoptosis Dimethylarsinic acid exposure also elevated intracellular HSP72 levels, changing the localization of the molecule and suppressing apoptosis of human alveolar cells (Kato et al. 2000). Ruicheng Hu et al, 2011 found that expression of HSP27 and CyPA was upregulated in smokers, and this upregulation was further marked in COPD smokers. HSP27 protects the lung cells of smokers and COPD patients against oxidative stress and apoptosis. HSP27 inhibits apoptosis by stabilising the mitochondrial electric potential and inhibiting the release of cytochrome C (Bruey et al, 2000; Paul et al, 2002). It promotes survival by activating the mitogen-activated protein kinase (MAPK) signal transduction pathway. Stress has been reported to activate the MAPK signal transduction pathway, including p38 MAPK, which induces expression and phosphorylation of HSP27 through MAPK-activated protein kinase 2 (MK2). So HSP27 inhibits both the intrinsic and extrinsic apoptotic pathways. It can inhibit the release of cytochrome-C or Smac-Diablo from mitochondria as well as act downstream of them preventing the formation of apoptosome. It can also act at the level of caspase-3 activation (Arrigo et al, 2007). At the level of the Fas receptor HSP 27 inhibits the extrinsinc signalling pathway by binding to DAXX (Arrigo et al, 2007). HSP27 is responsible with the Akt signalling pathway and also inhibits its activity (Arrigo et al, 2007). Concerning the structural organization of HSP27 in apoptotic cells it seems that its chaperone activity largely correlates with its anti-apoptotic one as far as in apoptotic cells the large oligomers inhibit caspase activation.αB crystalline also excutes protection against a large panel of apoptotic stimuli. It binds proapoptotic Bax, Bcl-xl and p53 polypeptides and prevents their translocation to mitochondria. It also directly inhibits the proteolytic activation of pro-caspase 3 (Mao et al, 2004; Liu S et al, 2007).

4. Inflammation, COPD and heat shock proteins

Chronic obstructive pulmonary disease is a slowly progressive condition, characterized by airflow inflammation, which is largely irreversible. It is suggested that the main etiological factor - cigarette smoking, produces inflammatory response in the lungs of all smokers and those who develop COPD have an abnormal or enhanced inflammation

4.1 Intracellular heat shock proteins – Antinflammatory molecules

The heat shock response is one of the most evolutionary conserved protective mechanisms in cells. It involves a temporary modification of gene expression. Synthesis of different heat shock proteins helps the organism cope with environmental and physiological stresses. As anti-inflammatory effector the heat shock response modulates signal transduction and gene expression by inhibiting the translocation of transcriptional factor – nuclear factor kappa B to the nucleus and prevents the expression of inflammatory mediators (Wong et al, 1997; Sun et al, 2005; Malhotra and Wong, 2002). Initial observations in animals linked heat shock response to an altered inflammatory response and demonstrate that heat preconditioning confers survival in otherwise lethal endotoxin stress (Snyder et al, 1992; Ensor et al, 1994). Heat conditioned macrophages show decreased secretion of TNF-alpha induced by endotoxins. This decreased secretion was sustained as long as the cells had elevated HSP70 levels. Similar to these studies endotoxin induction of IL-6 was also unchanged in the heat-

conditioned cells. The decrease of cytokine production was associated with a decrease in cytokine mRNA, suggesting that cell stress response regulates cytokine transcription. (Snyder et al, 1992; Ensor et al, 1994)

The liver has been the most extensively studied tissue to delineate the heat stress response in animals, because of the massive accumulation of HSP70 following stress. There is compelling data to support a direct link between liver HSP70 accumulation and altered survival and inflammatory cytokine profile seen in heat-conditioned animals undergoing endotoxin stress. Human peripheral blood macrophages overexpressing HSP70 also inhibited lipopolysaccharide induced production of TNF-α, IL-1β, IL-10 and IL-12 (Ding et al, 2001; Dokladny et al, 2008). These data taken as a whole demonstrates, that HSP70 expression is sufficient to alter proinflammatory cytokine production and increase endotoxin tolerance. A number of potential explanations have been suggested for the HSP mediated inflammatory repression. Immune cells are stimulated by a number of incoming signals from cell surface, including ischaemia, oxidative stress, LPS etc and initiate an inflammatory response by the activation of signalling pathways and transcription factors. The NFκB transcription factors play a pivotal role, altering the expression of cytokines, chemokines, cell adhesion molecules, growth factors, anti-apoptotic proteins and immunoreceptors (Brasier et al, 2006). Inactive NFκB is normally found in the cytoplasm, bound to its inhibitory protein, IκB. NFκB is activated by a number of incoming signals. These activate IKK, which phosphorylates IκB and allows NFκB to translocate into the nucleus and bind its target genes – TNFα, IL-1β, IL-6, IL-12 (Zhang and Ghosh, 2000).

HSP70 interacts directly with the NFκB inhibitor protein – IκB-α, which appears to prevent NFκB dissociation. HSP70 blocks IκB-α degradation and up-regulates IκB-α mRNA. Another mechanism of inflammatory suppression is indirect mechanism - repression of MAPK activation that mediates the inhibition of NFκB cascade. In addition, HSP70 suppresses activation of Jun kinase (JNK) MAPK. This prevents the phosphorylation of c-JUN and the subsequent activation of transcription factor AP-1. (Wang et al, 2002)

High mobility group box 1 protein (HMGB1) can trigger MAPK pathway and subsequent NFκB mediated synthesis and release of inflammatory mediators. HMGB1 is another step in the inflammatory cascade that is regulated by heat shock response. (Tang et al, 2005). HSP 70 overexpression suppressed the release and translocation of HMGB1.

The anti-inflammatory function of the small heat shock proteins is also confirmed. They interfere with the signallingpathway through their ability to protect against oxidative stress (Mehlen et al, 1995) and through modulation of TAK-1 activity (Alford et al, 2007). Another mechanism is the ability of HSP27 to suppress NFκB activation by interaction with IKK-α and IKK-β (Kammanadiminti SJ and Chadee K, 2006). HSP27 is also needed for the activation of TAK1 and downstream signaling by p38 MAPK, JNK (Alford et al, 2007). Both HSP27 and αB-crystalline have crucial roles in the control of inflammatory processes.

Among the pathologies where anti-oxidative defence of HSP27 is important is the airway inflammation in asthma. It is observed that in the airway of asthmatic patients HSP27 has increased expression and generates protection against oxidative stress, accompanying the chronic inflammatory state of this tissue.

In addition to altering of cytokine production heat shock proteins are also capable to influence cell tolerance to cytokines (Kusher et al, 1990, Jattela et al, 1993). The mechanisms

by which HSP confer protection from cytokines are not clear but may involve the interplay of intracellular triggers related to cell survival, stress tolerance and inflammation. It is majorly accepted that this is cell type specific and is executed by the regulation of apoptosis. (Schett et al, 2003; Ran and Lu, 2004)

4.2 COPD and intracellular heat shock proteins – A mechanism of self-defence, a trigger for immune inflammation, or a chaperonopathology

There are a few studies that deal with the levels of expression of heat shock proteins in lung of COPD patients.

Gal et al, 2011 showed for the first time that cigarette smoke extract stimulates the expression of HSP72 in alveolar epithelial cells. One possible mechanism for this could be the activation of cell preservation mechanisms, including decreased degradation of HSP72 in cells exposed to severely damaging substances. Heat shock protein induction is cytoprotective by preventing the onset of apoptosis. HSP72 has been shown to protect cells both from apoptosis and necrosis (Fekete et al. 2006; McConkey 1998). There are studies confirming other noxa - dimethylarsinic acid exposure-elevating intracellular HSP72 levels, changing the localization of the molecule and suppressing apoptosis of human alveolar cells (Kato et al. 2000).

In addition HSP72 siRNA abolished the mRNA and protein increase in cells, in parallel apoptosis increased and less cells survived. These results confirm upregulation of HSP72 in the presence of CSE in order to ensure cell survival, and indicate key protecting role for HSP72 under this cellular stress condition. Moreover these authors show that the anti-apoptotic effects of dexamethasone in alveolar epithelial cells are accomplished only after the upregulation of HSP72 that follows the cigarette smoke exposure. According to these experiments, increase in the inducible form of HSP70(HSP72) following CSE administration might enable proper action of administered dexamethasone, by increasing the assembly of GR and opening the steroid binding cleft. Glucocorticoid receptor (GR) function is dependent on the HSP90/HSP70 chaperone machinery. Initial GR interaction with HSP70 appears to be critical for the triage between HSP90 heterocomplex assembly and preservation of receptor function. HSP70 is required for the assembly of protein - HSP90 heterocomplexes, and the two chaperones interact directly with each other while opening the steroid binding cleft in the GR (Pratt and Toft 2003).

The results of Gal et al, are consistent to those reported by Chao-Jun Li et al, 2007. They found upregulation of HSP70 in human lung fibroblasts exposed to cigarette smoke. Similar is the data represented by Doz et al, 2008 who report that there is an increase in the levels of HSP70 in bronchoalveolar lavage of mice exposed to cigarette smoke. The inducible form of HSP70 is also upregulated in monocytes and endothelial cells of COPD patients. (Balsano, et al, 1999; Ning et al, 2004)

Ruicheng Hu et al, 2011 applied proteomics (MALDI-TOFF) to compare the expression profiles of proteins in cell lysates of lung tissue of 24 COPD smokers (6 in stage I and 18 in stage II, stable COPD), 24 non-COPD smokers, and 24 never-smokers. Age, gender distribution, body mass index did not differ significantly between the groups. Smoking index did not differ significantly between 11 COPD smokers and non-COPD smokers. Key spirometric parameters, including FEV1, FEV1%, FVC%, maximum predicted expiratory

flow rate at 50% of vital capacity (MEF50%), and MEF25% were comparable between the non-COPD smokers and never-smokers.Twenty-four proteins were identified by MS as being differentially expressed among the three groups of subjects. The main functions of these proteins involve basic metabolism, oxidation/reduction, coagulation/fibrinolysis, protein degradation, signal transduction, inflammation, and cell growth/ differentiation/ apoptosis. Proteomic analysis revealed that the expression of HSP27 and CyPA was upregulated in smokers, and this upregulation was particularly marked in COPD smokers. The variation in expression of HSP27 and CyPA between the groups was confirmed by IHC and Western blotting. Based on the results from the present study and other studies that have shown a protective role for HSP27 against oxidative stress and apoptosis, it is suggested that induction of HSP27 protects the lung cells of smokers and COPD patients against oxidative stress and apoptosis.

In contrary to the other heat shock proteins Capello et al, 2006 found a decrease of tissue expression of HSP60 and HSP10 that were parallel to chronic obstructive pulmonary disease progression, but did not correlate to the severity of COPD in smoking patients with NSCLC. They detected a trend for a decrease of intracellular expression of this chaperone that correlated best to the degree of tissue dedifferentiation.

In conclusion most of the studies dedicated to the role of heat shock proteins in COPD pathology are concentrated on tissue cell lysates or epithelium. The up-regulation of chaperones in them seems to be a protective mechanism, providing survival through anti-apoptotic and anti-oxidant role. Little is known about the expression of these cell proteins in neutrophils, lymphocytes and dendiritc cells and the way they influence the immune inflammation.

4.3 Extracellular heat shock proteins – Pro-inflammatory molecules

Intracellular HSP confer anti-inflammatory state because they downregulate inflammatory cytokine production, increase cellular tolerance to cytokines mediated cytotoxicity and attenuate epithelial barrier permeability changes. Alongside heat shock proteins are involved in multifaceted inflammatory reactions when seen by immune effector cells in the extracellular environment. They serve as co-stimulator molecules for immune recognition and are among the major molecules, refered by Matzinger, as "danger" signals (Matzinger, 2002) The release of HSP in the extracellular environment is an area of current research. It is known however that HSP can be released either passively after cell necrosis, trauma of cells or viral infection, or actively – exercise, physiological stress, certain diseases (Basu et al, 2000, Caldwerwood et al, 2007; Hightower et al, 1989). In addition the immune effects of HSPs are different when released by necrotic cells or during physiological response. The immunogenic activity of HSPs is mediated by two main mechanisms: 1) cytokine reponse by the activation of Toll-like receptors 2) facilitation of antigen uptake and formation of HSP-peptide complexes by antigen presenting cells. (Asea, et al, 2007)

Extracellular HSP can bind to cell surface receptors like TLR2 and TLR4 and lead to signaling events and activation of antigen presenting cells (Srivastava et al,1994). This activation leads to a signalling cascade and includes the activation of IL-1 and NFκB signal transduction pathway. HSP70 for example signals through TLR2 and TLR4 with the involvement of CD14 of human monocytes. This is followed by an upregulation of inflammatory cytokine secretion (IL-1β, IL-6, TNF-α).

Extracellular HSP, particularly HSP70, HSP90 and gp96, serve as antigen carriers and facilitate antigen uptake by dendritic or antigen presenting cells. Uptake is mediated by several mechanisms, including the α2-macroglobulin receptor (Binder et al, 2000). The HSP-peptide complex is more efficiently taken up by APCs than antigen alone. In addition HSP also stimulate APC maturation and activate NFκB signal pathways (Basu et al, 2000).

HSP facilitate antigen processing and transfer to MHC – I complex for presentation to cytotoxic T-lymphocytes. They are also expressed on the surface of tumor cells in cell culture as well as in cells infected with viruses.(Multhoff et al,1997) The HSP expression on tumor cells correlates to direct natural killer cell induced cytotoxicity and can be blocked by incubating target cells with antibodies directed against HSP70 prior to NK cell exposure. (Roigas et al, 1998)

4.4 COPD and extracellular heat shock proteins

The molecular mechanisms by which cigarette smoke causes the inflammatory process and pathology of COPD remain poorly understood. Chronic bronchitis and lung emphysema are pathologic characteristics of COPD and both conditions result from progressive and amplified inflammation that destructs and remodels parenchyma. Immune activation is not restricted to the lungs and is systematic. It procedes even after smoking cessation. In contrast to other inflammatory conditions the inflammation in the lungs is severe even in the advanced stages. There is enough evidence that make it reasonable enough to hypothesize that COPD has some kind of autoimmiunity in its pathogenesis. Nowadays it is largely accepted that the oxidative stress, accompanying COPD leads to changes in cell structures that makes them antigenic, thus triggering an autoimmune inflammatory response in patients with a genetic susceptibility.

It has been demonstrated that expression of HSPs is upregulated under stressful events in the lungs. Besides known intracellular chaperoning, it is possible that HSPs may also be released into the extracellular space following massive trauma or stress. This spillage of proteins serves as "danger signal" leading to cytokine transcription and release.

The involvement of extracellular HSP in COPD inflammatory process was described by Chao-Jun Li, et al 2008. They show that lung fibroblast exposed to cigarette smoke extract (CSE) release IL-8. The secretion was HSP70 dependent. Marked induction of HSP70 was observed in fibroblast culture medium in response to CSE. Upon exogenous administration of recombinant HSP70 to CSE treated fibroblasts, IL-8 production also increased. These results suggest that HSP70 is secreted into the extracellular environment via an unidentified mechanism that stimulates the production of IL-8 in primary lung fibroblasts. To examine whether it is the extracellular HSP70 that leads to CSE-induced IL-8 production, they determined CSE-induced IL-8 production in the presence or absence of neutralizing antibodies against HSP70 in the medium. Fibroblasts subjected to neutralizing antibody to HSP70 in the medium, exhibited marked reduction of CSE induced IL-8 production but did not completely abrogate the response. These data suggest that the extracellular HSP70 plays a critical role in mediating CSE-induced IL-8 production but also point to an HSP70-independent pathway of IL-8 production by CSE stimulus. They identified a novel early molecular pathway that mediates chemokine IL-8 release by human primary fibroblasts after cigarette smoke exposure. Early growth factor -1 (EGR-1) can trigger the synthesis of

HSP70. The HSP70 is then secreted into the extracellular environment and activates proinflammatory molecule (such as IL-8) production. They hypothesize that released HSP70 may activate cells through TLR-2, TLR-4, and CD14 and thereby mediate inflammation.

Similar are the results presented by Chase et al, 2007. Through their investigation, they have found evidence that supports the presence and biological activity of extracellular HSP72 in the lung. Chase et al, established that the airway epithelium itself is responsive to extracellular HSP72 and that this cytokine response is regulated through the TLR4 and NF-κB pathways. Their data would suggest that extracellular HSP72 is responsible for inducing and propagating inflammation, a process that is central to the pathogenesis of lung injury. Ganter et al. found extracellular HSP72 to be a marker of improved alveolar fluid clearance and therefore recovery from lung injury. This and other clinical investigations presenting divergent effects of extracellular HSP72 would suggest that the mere presence of HSP72 in the extracellular milieu is not the only factor. Chase et al, hypothesize that there may be a threshold of extracellular HSP72 that is required to maintain adequate signaling, below which the cells are unprepared for the insult, and above which excessive inflammation and therefore increased injury occur.

Doz et al, 2008 show that cigarette smoke exposure of the airways induces acute inflammation in mice. They found that airway inflammation is dependent on Toll-like receptor 4 and IL-1R1 signalling. Cigarette smoke induced a significant recruitment of neutrophils in the bronchoalveolar space and pulmonary parenchyma, which was reduced in TLR4(-) and MyD88 (-), and IL-1R1-deficient mice. Diminished neutrophil influx was associated with reduced IL-1, IL-6, and keratinocyte-derived chemokine levels and matrix metalloproteinase-9 (MMP-9) activity in the bronchoalveolar space. Cigarette smoke condensate (CSC) induced a macrophage proinflammatory response in vitro. The process was dependent on MyD88, IL-1R1, and TLR4 signaling, but not attributable to LPS. Heat shock protein 70, a known TLR4 agonist, was induced in the airways upon smoke exposure, which probably activated the innate immune system via TLR4/MyD88 an resulted in airway inflammation.They concluded that acute cigarette exposure results in LPS-independent TLR4 activation. This led to the IL-1 production and IL-1R1 signaling, which is crucial for cigarette smoke induced inflammation leading to chronic obstructive pulmonary disease.

Considering the presented data little is known about the clinical significance and mechanisms of secretion of extracellular heat shock proteins in COPD. There is scarce information about their relevance to the initiation and maintenance of the persistent inflammation.

4.5 Extarcellular heat shock proteins – A diagnostic marker, a marker of inflammation or disease severity

Hacker at al, 2009 investigated serum levels of heat shock proteins - HSP 27, 60, 70, 90alpha, 20S proteasomes, C-reactive protein (CRP), and interleukin-6 (IL-6). Serum levels were evaluated in healthy non-smoking volunteers (15), smokers without COPD (14), patients with mild to moderate COPD (19) and patients with severe or very severe COPD (16) were evaluated in four study groups. HSP27, HSP70 and HSP90α were significantly altered in patients suffering from COPD as compared to controls.

There was a significant increase of HSP27 in serum samples taken from the peripheral blood flow of patients suffering from COPD as compared to healthy smokers. Hacker et al, demonstrate a continuous increase of serum HSP27 concentrations with disease severity in their study. This effect may be due to increased tissue devastation especially in late stages of COPD and spreading of the inflammatory disease to other organs provoking a systemic spillage of HSP27 into the vascular bed. HSP27 generally acts as antiapoptotic mediator and can be seen as an endogenous immunosuppressive attempt to control excessive inflammation in COPD. Serum contents of HSP27 showed diagnostic potential to determine the occurrence of COPD in a logistic regression model and may serve as a marker for diagnosis and prediction of disease severity. Serum levels of HSP70 were elevated in patients at early and late stages of COPD. There was a four-fold increase in the GOLD I-II group compared to non-symptomatic smokers. Values were highest in the COPD I-II group, indicating a state of vast immune activation primarily at the early stages of the disease. Serum levels of HSP70 showed high sensitivity and specificity to define the occurrence of COPD in a logistic regression model and could serve as diagnostic marker. Because there was no significant difference between the COPD groups, HSP70 in comparison to HSP27 is unlikely to be a suitable marker for disease progression or response to therapy.

Rajagopal et al. characterized HSP90 as central factor in antigen presentation to T lymphocytes via major histocompatibility complex class II molecules (MHCII). Hacker et al, found soluble HSP90α was significantly upregulated in the peripheral blood of COPD groups as compared to healthy non-smokers. They hypothesize that elevated levels of extracellular HSP90α in COPD are essential in the adaptive immune system, triggering a possible autoreactive response to self-antigen. They suggest that HSP90α has immunomodulatory effects through cross-presentation of associated peptides in the context of major histocompatibility complex molecules. According to their results HSP60 is not a key element in the pathogenesis of COPD. Serum concentrations of HSP60 did not correlate with levels of other HSPs. Authors did not analyse whether there was a correlation between extracellular HSP and markers of inflammation – IL-6, CRP. They concluded that there were elevated serum concentrations of soluble heat shock proteins 27, 70 and 90α in patients with COPD. Their spillage into the vascular bed may be caused by continuous activation of the immune system in the deterioration of COPD through endogenous and exogenous trigger mechanisms. Furthermore, HSP27 and HSP70 showed statistical trends to serve as diagnostic markers or markers for disease progression.

Cherneva et al, compared the plasma levels of αB-crystalline in 63 COPD patients, 52 healthy-smokers and 48 smokers with inflammatory lung diseases. ELISA was applied as a method of detection. 43 of the COPD patients had severe disease (GOLD – III-IV) and 20 had mild disease (GOLD – I-II). The age distribution between the three groups was similar with a mean age of 67.24 (±8.06). All the patients had a comparable smoking exposure – 29.58 (±12.28) pack-years. In 26 of the COPD patients plasma levels of MMP-9 and CRP were also evaluated.

The mean levels of αB-crystalline were respectively: COPD patients – 0.352 (±0.12); healthy smokers – 0.291 (±0.07); smokers with inflammatory lung diseases – 0.433 (±0.27). Statistically significant difference was established between the COPD patients and the healthy smoking volunteers - (p=0.010) and between smokers with inflammatory lung

diseases and the healthy smoking volunteers (p=0.007). In comparison there was not a significant difference between the COPD patients and those with inflammatory pulmonary diseases - (p=0.158). No relation was established between αB-crystalline plasma levels and hsCRP and MMP-9 levels respectively (p=0.91 and p=0,76)

Authors concluded that αB-crystalline is not a specific diagnostic marker. It rather reflects oxidative stress, and inflammation that accompanies it.

5. COPD – An accelerated lung ageing disease, chaperonopathy or proteinopathy?

Ageing implies a certain period; the passage of time from conception till death. Senesence is a function of ageing and implies the molecular and cellular processes that accompany it. Senesence is the structural characteristics that appear as times goes by. They could either be physiological, or more often deleterious ones that occur in the organism after it has fully developed and affect its molecules, cells and tissues. A distinction should always be made between senescence and ageing, since the structural characteristic at molecular level do not always correspond to the age, depending on the "stress" conditions and the adaptive capacities of the organism.

Different organisms, particularly their cells are challenged to thrive to different environmental and emotional stress factors (physiological stress, emotional stress, oxidative stress, food deprivation, sleep deprivation, hypoxia, ischaemia, etc) for one and the same period of time. They are trying to keep the balance, reaching the homeostasis and getting adapted to the new conditions that they are currently in. This is performed by the stimulation and engagement of a number of evolutionary developed and genetically predetermined pathways:1) inflammatory pathways; 2) unfolded protein response 3) heat shock response 4) ubiquitin proteasome system. The pathways are executed by an armamentarium of chaperones that " take care" and provide functionally available proteins. In case of failure the damaged proteins are cleared by another armamentarium that clear the cells from destructive components.

Chaperonology, the study of chaperones and HSP is emerging as a new area of physiology and molecular biology that could be of importance in both pathology and medicine. Defective chaperone function can contribute to the etiology of pathologic conditions, known as chaperonopathies. Chaperonopathies can be genetic or acquired. Usually the latter are described as quantitative variations in chaperone levels in tissue or body fluids that are accumulated as a result of posttranslational modifications. These variations and modifications have been described for the separate members of the HSP and their association with old age and age-related diseases have been largely reported. However data is scarce to demonstrate neither a direct link between a chaperonopathology and a definitive molecular or cellular characteristic, typical of senescence, nor to attribute certain chaperonopathy to a specific disease, associated with ageing.

Age-dependent damage to proteins is one of the primary molecular features of senescence. The appearance of age-related post-translational modifications of proteins – proteinopathies can seriously disturb or entirely change their cellular function, or make them immunogenic. The accumulation of modified proteins as time goes by could be attributed to several models:

- A cell possessing a normal set of chaperones could potentially counteract the proteinopathies. However chaperones are themselves modified by the passage of time So it could be that both chaperonopathies and proteinopathies start in parallel and independently of one another. In that case chaperones would also play a major role, though not the primary one, demonstrating a failure of the cellular adaptation to maintain the protein homeostasis and functioning during senescence (age-related emphysema);

- There could be an accumulation of damaged proteins due to high levels of stress, which would result in a widespread deficiency of otherwise normal chaperones and thus lead to an accelerated onset of degenerative or age-related diseases. In that case chaperonapathies seem to be a major trigger in the mechanisms of senescence (COPD);

- Another possibility – the presence of genetically defective chaperone system a chaperonopathy that can not counteract and keep the protein homeostasis even when there are no environmental challenges. Chaperone failure in that case would cause the initiation and progression of proteinopathies. Besides this the abnormal chaperones would not perform their physiological roles in cells, thus affecting essential cellular processes (emphysema in COPD);

- Chaperonopathies could be treated as a different characteristic of the process of senescence but not one of its mechanisms. However taking into account clinical, pathological and experimental studies this is hardly reasonable.

Ignoring the dilemma, whether the chaperonapathies or the proteinopathies are first during the process of ageing, the immune system will inevitably response to what is going on in the organism. If this is the chaperonopathy 1) Chaperones could be spilled extracellularly as "danger" signals, activating the antigen-presenting cells; 2) chaperones could be associated with other peptides on the surface of cells announcing for a ''danger''cytotoxicity or; 3) chaperones can simply allow the accumulation of modified proteins that are accepted as self-antigenic.

If this is simply a proteinpathy as Kirkow assumes the defects cause inflammatory reactions, which can themselves exacerbate existing damage, so that inflammatory and antiinflammatory factors can play a part in modulating the outcome of the ageing process. Thus, age-associated inflammation/structural change is a failure of elimination and/or failure of repair (DNA, protein).

Ito and Barnes, 2009 define COPD as accelerated lung ageing disease. They find a lot of similarities between aged lung and COPD lung. These are not mere clinical characteristics – accelerated decline of lung function but a number of molecular ones.

1. Telomere length has been demonstrated to be significantly shorter in patients with emphysema than in asymptomatic nonsmokers. This is confirmed in alveolar type II cells and endothelial cells, (Tsuji et al, 2006) peripheral blood mononuclear cells,(Morla et al, 2006) and fibroblasts.(Muller et al, 2006)

2. There is ample evidence that oxidative stress plays a major role in COPD. Increase in nitrotyrosine deposition is also a feature seen in COPD lung as well as aged tissue. This is the evidence of an increase in nitrative/oxidative stress, and may contribute to the accumulation of nitrated and oxidized proteins. Superoxide dismutase enzyme activity is reported to be lower in long-term healthy smokers and in stable COPD patients than

in healthy adults,(Kirkril et al, 2008) although this is still controversial (Nadeem et al, 2005).COPD patients also have reduced total antioxidant capacity. Furthermore, ferric-reducing antioxidant power is lower in COPD patients, and it had a positive correlation with the severity of airways obstruction (FEV1 percentage of predicted).

3. There are several similarities in inflammation between ageing and COPD, such as neutrophil accumulation, NF-kB activation (Barnes, 2006) and increase in IL-6/IL-8/TNF-α. COPD patients are also corticosteroid insensitive as similar to healthy aged people. Protein turnover system in COPD is also impaired. HDAC2 is markedly reduced in COPD. This reductionis involved in enhancing inflammation is involved in enhancing inflammation and corticosteroid insensitivity.

4. Furthermore, expression of antiageing molecules are reduced in COPD - SIRT1 is a major inhibitory regulator of MMP-9, and reduction in SIRT1 causes structural changes of lung, such as emphysema (Vuppusetty C, et al 2007; Rajendrasozhan S, et al. 2008). SIRT6 loss leads to abnormalities in mice that overlap with ageing-associated degenerative processes, and SIRT6 is a nuclear, chromatin-associated protein that promotes resistance to DNA damage (Meyer et al, 1988).

Analysing the presented data we can assume that COPD could be regarded as a chaperonopathy (proteinopathy), as a model of accelerated ageing, in which the organism can not keep the homeostasis under the conditions of oxidative stress. The immune system is involved, but instead of restoring the balance it augments the oxidative stress, generating a large number of reactive – oxygen species. This leads to the accumulation of modified self-proteins that are recognized as antigenic. Autoimmunity occurs as an epiphenomenon. A vicious circle is created. The environmental and the inflammatory oxidative stress leads to the accumulation of modified proteins. Chaperones are additionally depleted or also modified. Ubiquitin proteasome system is overloaded or also modified.

6. COPD as a risk factor of lung cancer

COPD is currently the leading cause of morbidity and mortality worldwide whose prevalence and burden are projected to increase due to smoker exposure and the changing age structure of the world population, particularly in women (Lopez et al, 2003). The presence of COPD increases the risk of lung cancer of up to 4,5 fold among long-term smokers. COPD is by far the greatest risk factor for lung cancer amongst smokers in 50-90% of smokers with lung cancer (Young et al, 2009). Even a small reduction of FEV1 is a marker of airflow obstruction and is a significant predictor of lung cancer (Wasswa et al, 2005).

Lung cancer accounts for 12% of cancers diagnosed worldwide, making it the most common malignancy other than non-melanoma skin cancer. Approximately over one million die of lung cancer each year (Jemal et al, 2009). Worldwide, tobacco smoking is associated with more of 90% of cases of lung cancer. In less developed countries lung cancer rates are predicited to continue to increase due to endemic tobacco use. In more developed countries, the incidence and mortality rate are generally declining, reflecting previous trends in smoking prevalence (Youlden et al, 2008). Only 15% of life-time smokers develop lung cancer and 10% of lung cancers occur in never smokers especially in women and in Asiatic women in particular, which underlines the role of genetics (Scagliotti et al, 2009).

Lung cancer is also a leading cause of morbidity and mortality in patients with COPD as 33% of patients died of lung cancer over a 14,5 year follow-up (Anthonisen et al, 2005; Yao et al, 2009). Furthermore ≈60% of patients, diagnosed with lung cancer have a spirometric evidence of COPD (Molina et al, 2008). NSCLC accounts for 85% of lung cancer cases in USA and the COPD related cancer type (squamous cell lung cancer) still represents the most common histological subtype of lung cancer in European men (Papi et al, 2004). Despite significant advances in diagnostic approaches, the pathology of lung cancer is still elusive and there has been little improvement in 5-year survival rates (≈ 15% overall; <14% among males and <18% among females) (Youlden et al, 2008).

Two of the leading causes morbidity and moratlity worldwide – COPD and lung cancer are due to the environmental risk factor and cigarette smoke exposure in combination with genetic predisposition.

6.1 HSP and cancer

There is a cascade of molecular events that mediate the transformation of a normal cell to a cancerous one. Several etiological factors and events are recognized as triggering mechanism of cancerogenesis – viruses, radiation, hereditary and non-hereditary mutations, carcinogenic compounds. Most tumors are formed by stepwise progression of normal cell into a cancer one by using alterations in cell physiology, described by (Hanahan and Weinberg, 2000) – self sufficiency in growth signals, insensitivity to growth inhibiton, evasion from apoptosis, limitless replicative senescence, sustained angiogenesis and tissue invasion and metastasis. Heat shock reponse participates in cancerogenesis of both up – and downregulation of specific heat shock proteins. Variations in HSP expression could be found in many tumors and preneoplastic lesions as well. At a histological level the transition from a normal tissue to tumor is accompanied by the increase in HSP expression. HSP are involved in the cancerogenesis and are up-regulated to protect cells from apoptosis and induce drug resistance. Their role in cancer cells is to protect other proteins against aggregation, to solubilize initial protein aggregates, to assist in folding of nascent proteins or refolding of damaged proteins; to target severely damaged proteins to degradation. Overexpression of HSP in cancer cells is beneficial to their survival because they inhibit apoptosis and induce drug resistance. They act as a double-side sword. Some of them - HSP90 maintains chaperoning function in a number of oncogenic molecules and promotes tumor survival. Others – HSP60,70 and HSP72 may sensitize cancer cells to immune attacks by two mechanisms. They may be expressed on tumor cell surface and enhance their recognition by NK-cells or induce antitumor immunity by HSP related tumor vaccines (Calderwood et al, 2006).

6.1.1 HSP and non-small cell lung cancer

Bonay et al, 1994 are the first to study the expression profile of HSP in the normal lung as well as the effect of cigarette smoke on their expression. They provide detailed description of HSP distribution in lung carcinoma, applying both immunohistochemical and immunoflourescent techniques for their investigation. In lung tissue from non-smokers, lung epithelial cells are positive for HSP90 and the inducible form of HSP70. There was also a weak expression of HSP60. Macrophages also expressed these HSPs but weaker in

comparison to bronchial epithelium. However no other parenchymal, immune or inflammatory cell was positive for these heat shock proteins.

Cigarette smoking modifies neither the distribution, nor the intensity of staining in bronchial epithelium in smokers. Macrophages also expressed one or more of the HSP but in low levels. Bonay et al, 1994 have shown considerable heterogenity in the expression of HSP by cells in a given tumor. They explained their observations by different degree of differentiation state of the cells.

HSP90 is required for conformational maturation as well as stability of many proteins, involved in signalling pathways. It is responsible for the functional activity of a lot of oncogenic kinases that drive the signal transduction and proliferation of lung cancer cells. It seems to be upregulated in lung cancer and recently it has been connected with the stabilization of the mutant form of EGFR and one of the mechanisms for the resistance to tyrosine kinase inhibitors.(Shumamura et al, 2005, 2008)

HSP70 is another chaperone of interest in lung cancer. Volm et al, 1995 studied the resistance of lung cancer and its associatation to HSP70 expression. Tumor samples of 90 patients with NSCLC were investigated by immunohistochemistry, and no association between HSP70 and doxorubicin resistance was found. However a trend for an association between glutathione-S-transferase positivity and HSP70 positivity was observed. In addition there was a strong positive association between catalase positivity and HSP70 positivity. These observations show that both heat shock and stress promote intracellular oxidative damage and catalases are necessary for their protection.

Malusecka et al, 2001 studied the expression profile of both HSP70 and HSP27 proteins in 106 patients with NSCLC. They found in the majority of patients (95/106) both cytoplasmic and nuclear positivity to HSP70. In stage I tumors and dysplastic lesions however there was an enhanced nuclear positivity. As for HSP27 a positive cytoplasmic immunostaining in 70% of cases with the highest score for squamous cell lung cancer was found. A positive association for the expression levels of both proteins was also described. HSP27 and HSP70 were indicated as important factors for lung tumor transformation process, as well as for factors for chemoresistance.

Capello et al, 2006 studied the role of HSP60 and HSP10 in lung cancerogenesis. They described the level of expression of these chaperones in 35 patients with spirometirically proven COPD and compared them to the levels of expression in 10 adenocarcinomas and adenosquamous cell lung cancers. In normal bronchial epithelium that was found in 10 of the COPD patients HSP60 and HSP10 were positive in 23% of cases. In basal hyperplasia lesions they were positive respectively in 29% and 26%. Only 3% of squamous metaplastic lesions were positive for HSP60 and only 2% positive for HSP10. Of the dysplastic lesions 3% were positive for HSP60; 2% - for HSP10. Adenosquamos cell lung cancer was negative for both chaperones. The authors showed that HSP60 and HSP10 loss is related to the development and progression of bronchial cancer in COPD patients.

Recently Jackson and Garcia Rojas investigated the role of HSP27 in cellular resistance in lung cells, and reported that HSP27, which is phosphorylated by MAPK pathway protect epithelial cells from oxidant stress.

The role of another small heat shock protein in NSCLC was described by Cherneva et al, 2010.The expression of alpha-B crystalline was explored applying immunohistochemical

analysis on a tissue microarray slide, containing samples from 146 NSCLC patients - 96 squamous cell lung tumours, 10 adenosquamous carcinomas, 35 adenocarcinomas and five broncho-alveolar carcinomas. Tumors were of different grade of differentiation (29 - well differentiated, 56 -moderate and 36 - poor differentiation) and different clinical stage - 37 patients were in stage I, 27 in stage II, 65 in stage III and 17 in stage IV.

αB-crystallin was not detected in the normal alveolar pneumocytes; a few of the peribronchial glands, however, stained faintly but only in the cytoplasm. Although partially, there were a few areas where basal epithelial cells of the normal ciliated bronchial epithelium also showed weak cytoplasmic staining and no nuclear staining. In contrast, the basal layer of the tumours showed intensive cytoplasmic staining and a lack of nuclear staining. Lymphoid cells infiltrating the tumour stroma as well as the macrophages showed no cytoplasmic staining, but the nuclear staining varied from intensive to a lack of staining. Apoptotic and necrotic cells had faint cytoplasmic and intensive nuclear staining. Intensive nuclear staining was also detected in cells undergoing mitosis.

Nuclear staining was detected in 133 tumours (95 squamous cell histology and 38 adenocarcinomas). Cytoplasmic staining was detected in 127 tumours (95 squamous cell histology and 32 adenocarcinomas). Lack of nuclear staining was detected in 44 (33%) cases and intensive nuclear staining was observed in 89 (67%). A total of 26 tumours strongly expressed αB-crystallin in both nucleus and cytoplasm. Most of the tumours showed homogeneous cytoplasmic staining; more than 60% of the cells of the tumour had the same intensity of staining. The heterogeneity was detected up to the level of nuclear staining. The cytoplasmic staining was not of statistically significant correlation to histology. In contrast, the nuclear staining proved to be characteristic for the adenocarcinomas ($p < 0.001$, Contigency Coeff Cramer 0.369).

αB-crystallin was significantly overexpressed in NSCLC. In these tumors, the cytoplasmic expression of αB-crystallin was statistically significantly related to the tumour size (T-factor). This might be due to the fact that αB-crystallin has been reported to serve as a chaperone under stress conditions for other oncogenic molecules (beta-catenin, cyclin D1 and VEGF) or is itself oncogenic (Ghosh J, 2007).

In comparison to breast, renal and colorectal cancers, where only cytoplasmic and membraneous staining was reported, in NSCLC a nuclear staining was observed. The nuclear relocalisation is a characteristic feature for the whole group of small heat-shock proteins and in most cases is triggered under stress conditions (Klemenz R et al 1991, Voorter Ch et al 1992). In the nucleus, αB-crystallin is claimed to be responsible for the stabilisation of the speckled architecture of lamin A/C and is thus involved in the splicing factor compartment (Adhikari A, et al 2004). IJssel et al, 2003 discuss that its fundamental role in the nucleus (transcription, splicing and genomic stability) is difficult to be discerned from its chaperone function in that cellular compartment.

The precise biologic function of both cytoplasmic and nuclear localisation of the protein is obscure and needs other approaches for elucidation. Moreover, the variability of cellular compartment expression is complicated by the fact that in many epithelial tumours the protein is down-regulated and lacks cytoplasmic expression – buccal cancer and head and neck cancer. The importance of αB in NSCLC may be due to the fact that the nuclear staining was characteristic for adenocarcinoma histology and was significantly related to the

tumour stage (p = 0.042). Patients whose tumours had nuclear staining had shorter overall survival time in comparison to those that lacked staining (log-rank test p = 0.002). This supports the hypothesis that the nuclear positivity of the tumours refers to a more aggressive tumour biology. The nuclear positivity of αB in NSCLC stratifies patients from II and III stage in risk subgroups. Keeping in mind that more than 75% of patients are diagnosed at stage III, the introduction and validation of prognostic markers at this stage would undoubtedly help in predicting recurrence and improving clinical prognosis.

To sum up the role of heat shock proteins in NSCLC we can say that the high molecular chaperones are important molecular mechanisms in lung cancerogenesis, probably contributing by their chaperoning abilities related to other oncogenic molecules - (HSP90,70) as well as by performing their role in apoptosis – (HSP60,70). They could be used in cancer treatment as their inhibition (HSP90) is associated with overwhelming of chemoresistance (Shimamura et al, 2005, 2008) – or their induction (HSP70) as a way of sensitizing tumors to chemo- and radiotherapy (Gehrmann, 2006) The small heat shock proteins are probably related to the regulation of apoptosis, cytoskeletal stability, chaperoning of antioxidant enzymes and prevention of oxidative stress. Their clinical significance is related to their applicaton as markers for chemoresistance - (HSP27), or risk stratification and survival (αB-crystallin).

6.1.2 HSP as cancer diagnostic markers in COPD patients

HSP were found to be expressed on the surface of tumor cell and extracellular HSP were reported in plasma of both cancer and non-cancer patients. As HSP are highly immunogenic their cell surface and extracellular expression was employed in the production of vaccines as well as an approach for cancer detection.

Zhong et al, 2003 first decribed the diagnostic significance of extracellular HSP70 and 90 in NSCLC patients. The assay was performed in a group of 49 NCSLC patients and 40 healthy volunteers. The diagnostic utility of the HSP70 expression showed a modest sensitivity 0.74 and specificity 0.73; area under the curve AUC=0.73; while HSP90 antibodies were of poor performance AUC-0.602.

Wang et al, 2010 also tried to characterize the levels of expression of HSP70 and HSP27 in plasma and lymphocytes. They compared the expression of these chaperones in 99 coal miners without NSCLC, 51 coal miners with NSCLC and 42 patients that were not coal-miners. They found higher levels of plasma HSP27 and HSP70 in coal miners, which corresponded to a higher risk for lung cancer. Lymphocytes of coal miners with NSCLC had the lowest levels of intracellular HSP70 compared to coal miners and non-coal miners.

The presence of αB antibodies in NSCLC patients was also reported. Cherneva et al, 2010 compared the levels of expression of αB-crystallin in 51 NSCLC patients, 38 high risk COPD patients and 52 age and sex matched healthy volunteers. They found that the expression of αB crystalline antibodies was significantly higher in NSCLC patients in comparison to age and sex matched healthy volunteers - (p<0.001). αB-crystallin antibodies showed sensitivity 62% and specificity 72% in discerning cancer patients among healthy volunteers.

The clinical significance of αB-crystallin antibodies however is limited while comparing the healthy volunteers to the high risk group of COPD patients. A potential explanation of this

could be that the major characteristic of this pathology is the increased oxidative stress and chronic systemic inflammation, predominantly localized in the lungs.This may provoke reactive αB-crystallin protein overexpression in COPD patients, as one of its function is antioxidation (Aggeli et al, 2008).

Analysing the levels of antibodies of αB-crystallin in the plasma of patients with NSCLC and their clinicopathological chracteristic, Cherneva et al, found no significance between pathological parameters and this biological marker. This however was not the issue when concerning the lymphogenic spread of the disease. The levels of antibodies in patients with lymph node metastases was higher compared to those without them. The reason for this remains elusive and requires further investigation. The ROC curve analysis showed decent characteristics in discerning patients with and without metastatic spread –AUC 0.667 (95%CI – 0.515-0.820) sensitivity- 60% and specificity –70% at a cut-off 0.381. It should be however carefully taken in consideration that the clinical staging does not envisage the molecular one and the presence of already spread micrometastatic disease in N0 patients is obscure. Whether the higher rate of antibodies in patients with lymph metastases corresponds to a better immune reactivation and host defence remains a matter of question, since patients should be followed up.

Summarizing, the expression of heat shock proteins in NSCLC patients is of limited significance as a diagnostic approach, either alone or in a combination panel. The presence of αB-crystallin antibodies in plasma of NSCLC patients seems to be due the reactivation of the immune system and is unspecific as far as it is provoked under various stress conditions. The higher levels of the antibodies detected in patients with lymphogenic metastatic spread could be of clinical application as far as they could be used as markers for risk of recurrence and patients' prognosis.

7. Future research in COPD

Although a lot of research is done, unraveling the signalling pathways and the intimate mechanisms of innate and adopted immunity, COPD remains a leading cause of morbidity and mortality worldwide. It seems that the current concepts of COPD are not leading to a solution that can be applied in clinical practice. It refers to both pathogenesis and treatment.

7.1 "Stressing" the oxidative stress, inflammation and autoimmunity

Oxidative stress does exist in the lungs and it is inevitable, having in mind its physiology. Keeping its balance is a kind of self-protection. COPD is obviously a disease of disbalance - oxi/antioxidant, protease/antiprotease, apoptosis/proliferation, acetylases/deacetylases. In most cases COPD develops in smokers that makes us think that it necessarily is related to smoking, but why only 20% of smokers develop COPD? Moreover how can we explain the presence of non-smoker 's COPD? Is this another disease? If not can we say that it is a proteinopathy? A proteinopathy that sets a disbalance in lung cells even in the absence of exogenous noxa; a reason for sending a "danger" signal to the innate immunity that acts on default and instead of helping additionally stresses the already "stressed cells". Could it be that COPD in smokers is also proteinopathy, but acquired? There are not enough studies that let us make certain conclusions about the role of chaperones, particularly of heat shock proteins in COPD. There is only data that let us make hypotheses.

Can we assume that: In smokers' lungs, cells are exposed to additional stress, which acquires the intensive assistance of chaperones to maintain their proteins functionally available. In some individuals the chaperone system cannot adapt to the new set-point. The balance is disturbed. The cells are "stressed" and send signals for help. At the same time, however, there is already a depletion of chaperones and accumulation of abnormal proteins that appear as a result of the disbalance (proteinopathy/chaperonopathy) and this is another reason for sending "danger signals" to the immune system. These "danger signals" could themselves be heat shock proteins as the studies present. The "danger signals" bind to Toll-like receptors, which according to Doz et al, are essential for triggering the inflammation in COPD. Neutrophils are sequesterd in the pulmonary circulation and themselves become "stressed" Obviously something "happens" to their cytoskeleton as they become less deformable and retained. It is highly probable that their small heat shock proteins could not restore the cytoskeletal damage. Their F-actin probably cannot reorganize and form the lamelipodia and fillopodia needed for migration. Thus instead of protectors, neutrophils become generators of oxidative stress but also of inflammatory mediators - that is their cell-type specific response to stress. The immune system is activated. An inflammatory response follows. The innate immunity is triggered. Dendritic cells are already activated through their Toll-like receptors and the adoptive cell response becomes engaged. By that time the accumulation of modified proteins is still going on, the chaperone system and energy balance are overloaded. Instead of resuming the misfolded proteins, HSP – peptide, self-antigenic complexes accumulate in cells. Autoimmunity probably appears as an epiphenomenon. Rather than being resolved the problem persists, accelerated by the induction of an autoimmune inflammation. Despite performing their own cellular functions chaperones are trying to keep the other proteins. The cellular physiology is disturbed and a lot of cells commit a suicide. This leads to the spillage of "danger molecules", modified proteins, and self-antigenic products, that additionally activate the immune system. Inflammation persists despite of the elimination of the primary noxa and is even augmented by the immune cells. The "stressed lung cells" are sending danger signals to the immune system but instead of altruistic response they are put under even severe stress. The disbalance between proteins - acetylase/deacetylase; oxidation/antioxidation; proteases/antiproteases – changes the phenotypic characteristic of lung cells, as well as their environment – tissue remodelling follows. The functional characteristics of lung cells is already irreversibly deteriorated.

It is highly probabale that COPD smokers have chaperonopathy, affecting their cells as a whole but as lungs are the primary organ proteomic analysis of lung cells lysates will probably help most unraveling the puzzle. Microdissection techniques on epithelial cells should be used in patients with GOLD-0 and be compared to GOLD- I-II, GOLD – III-IV. It would be more reasonable to select patients considering phenotypes in COPD.

It would be interesting to find the role of extracellular HSP70 in COPD etiology an progression. The ''danger signal'' that stimulates inflammation through Toll-signalling.

7.2 Exacerbations – A spillage of HSP, or antigenic mimicry?

There is a lot of data about inflammatory and autoimmune (neurodegenerative, degenerative joint diseases, atherosclerosis, diabetes type I) diseases triggered as a result of

cross-reactivity between bacterial HSP and human HSP. Assuming this we can hypotheisize that exacerbations in COPD may reflect antigenic mimicry – a cross-reactivity between the self-antigenic HSP-peptide complexes accumulated in lung cells and the evolutionary conserved HSP of the infectious viruses and bacteria. Determining the levels of HSP65 during exacerbations could give a clue.

Exacerbations could also be a massive spillage of extracellular heat shock proteins that trigger the Toll-like receptors and thus induce immune response.

7.3 Treatment

Despite being or not a chaperonopathy, it will certainly be of interest to know whether the chaperones within the heterocomplex (HSP90/HSP70/GR) of the glucocorticosteroid receptor are related to corticosteroid resistance and if so how could this be modified? How is HSP90 involved in the telomere assembly and how can be manipulated to deter the telomere shortening and lung ageing? If Toll-like receptors and danger molecules as HSP 70 are the primary triggers of the immune response in COPD lung how effective will be their inhibiting? If COPD is a chaperonopathy how can we booster the chaperones   instead of restoring the balance at so many levels - acetylase/deacetylase; oxidation/antioxidation; proteases/antiproteases? Is it reasonable to think that resuming one side of the problem as the application of sirtuin agonists, antioxidants, steroids would be a solution? Isn't it more reasonable to think of the natural protectors of these molecules that ''chaper'' (keep) them from alteration?

8. Conclusions

There is scarce data on the role of chaperones in the molecular pathogenesis of COPD. The contemporary techniques – proteomics (MALDI-TOFF) and transcritpomics (SAGE – serial analysis of gene expression) show overexpression of both high molecular weight – HSP70 and small heat shock proteins – hem-oxygenase -1, HSP27 in lung cell lysates. Far more studies are dedicated to the high levels of circulating extracellular heat shock proteins, representing them as a trigger for Toll-like receptor mediated immune response – HSP70, or as a panel of diagnostic markers in COPD – HSP90, HSP70, HSP27. Presuming the biological functions of the chaperone system, its significance in protecting cells from "stress", its large collaboration with the immune system and importance of preserving the proper functioning and balance of the proteome within cells it is undoubtedly necessary to further elucidate their place in COPD etiology and progression.

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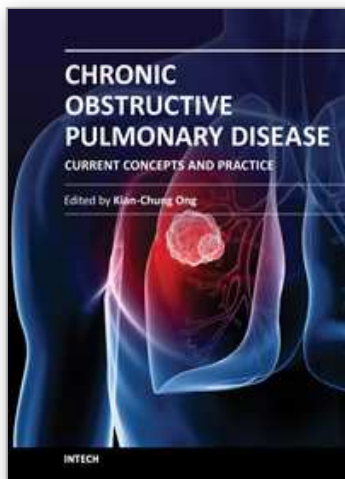
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A decade or so ago, many clinicians were described as having an unnecessarily 'nihilistic' view of COPD. This has certainly changed over the years... This open access book on COPD provides a platform for scientists and clinicians from around the world to present their knowledge of the disease and up-to-date scientific findings, and avails the reader to a multitude of topics: from recent discoveries in the basic sciences to state-of-the-art interventions on COPD. Management of patients with COPD challenges the whole gamut of Respiratory Medicine - necessarily pushing frontiers in pulmonary function (and exercise) testing, radiologic imaging, pharmaceuticals, chest physiotherapy, intensive care with respiratory therapy, bronchology and thoracic surgery. In addition, multi-disciplinary inputs from other specialty fields such as cardiology, neuro-psychiatry, geriatric medicine and palliative care are often necessary for the comprehensive management of COPD. The recent progress and a multi-disciplinary approach in dealing with COPD certainly bode well for the future. Nonetheless, the final goal and ultimate outcome is in improving the health status and survival of patients with COPD.

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