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Minireview

Hsp27 (HspB1) and αB-crystallin (HspB5) as therapeutic targets

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Abstract Hsp27 and αB-crystallin are molecular chaperones that are constitutively expressed in several mammalian cells, particularly in pathological conditions. These proteins share functions as diverse as protection against toxicity mediated by aberrantly folded proteins or oxidative-inflammation conditions. In addition, these proteins share anti-apoptotic properties and are tumorigenic when expressed in cancer cells. This review summarizes the current knowledge about Hsp27 and αB-crystallin and the implications, either positive or deleterious, of these proteins in pathologies such as neurodegenerative diseases, myopathies, asthma, cataracts and cancers. Approaches towards therapeutic strategies aimed at modulating the expression and/or the activities of Hsp27 and αB-crystallin are presented.

Keywords: Hsp27; αB-crystallin; Neurodegenerative diseases; Myopathies; Cancers

1. Hsp27 and αB-crystallin: old and new

Heat shock proteins (Hsps) or stress proteins have in common a stimulated synthesis in response to heat shock or when the environment of a cell becomes deleterious and alters protein folding. In cells exposed to heat shock, Hsps act as molecular chaperones that counteract the formation of aberrantly folded polypeptides and allow their correct refolding during stress recovery. In addition of being expressed in stressed cells, some Hsps show a basal level of constitutive expression and act as in-house chaperone towards several fundamental cellular processes, such as protein intracellular transport, cytoskeletal architecture, mutations masking, translation regulation, intracellular redox homeostasis or protection against spontaneous or stimulated programmed cell death.

Mammalian Hsp27 (HspB1) and αB-crystallin (HspB5) belong to the family of small heat shock proteins (sHsps). In human, 10 different sHsps have been characterized but only few of them, as Hsp27, Hsp22 and αB-crystallin, are true heat shock proteins that display an enhanced synthesis in response to stress. Up until now, the more studied sHsps have been mammalian Hsp27 and αB-crystallin. sHsps are characterized by low molecular masses (12–43 kDa) and a conserved C-terminal domain (the α-crystallin domain, see Fig. 1). sHsps also contain a WDPF domain in their N-terminal part and a non conserved flexible domain which constitutes the C-terminal part of the proteins. sHsps share the property to form globular oligomeric structures that are characterized, in mammalian cells, by molecular masses ranging from 50 to about 700–800 kDa. The dynamic organization of sHsps oligomers appears to be a crucial factor which controls the activity of these proteins. We still do not have a good knowledge of the structural organization of sHsps. This is mainly due to the heterogenous size and dynamic properties of sHsps oligomers and of their ability to form hetero-complexes with other members of the sHsps family. An intriguing property of some sHsps, such as Hsp27 and αB-crystallin, concerns their ability to be phosphorylated and therefore under the control of several transduction pathways. Indeed, both proteins show rapid phosphorylation that modulates their activities in response to a wide variety of stimuli [1,2]. Both proteins have phosphorylated serine sites in the N-terminal part of the polypeptides, in the WDPF domain [2] and close to the α-crystallin domain. Hsp27 is phosphorylated at serines 15, 78 and 82 by mitogen-activated protein kinases associated protein kinases (MAPKAP kinases 2,3) which are themselves activated by phosphorylation by MAP p38 protein kinase [3,4] (see Fig. 1). As Hsp27, αB-crystallin is phosphorylated at three serine site corresponding to residues 19, 45 and 59. At least two pathways are implicated in the αB-crystallin phosphorylation: the MAPKAPK2 kinases are responsible of the phosphorylation of serine 59 while serine 45 appears under the control of p42/p44 MAPKinase. The kinase responsible of the phosphorylation of serine 19 is still not known. Hence, Hsp27 phosphorylation can be modulated by signals as diverse as those mediated by growth factors, differentiating agents, tumor necrosis factor, oxidative stress or heat shock [5,6]. In the case of α-crystallin, a recent study has shown that disorganization of microfilaments or microtubules networks results in the activation of convergent pathways to MAPK p38 [1]. At least in the case of Hsp27, phosphorylation was demonstrated to result in a decrease size of the oligomers [7].

In addition of being overexpressed in stress conditions, Hsp27 and αB-crystallin share the ability of having a tissue/cell

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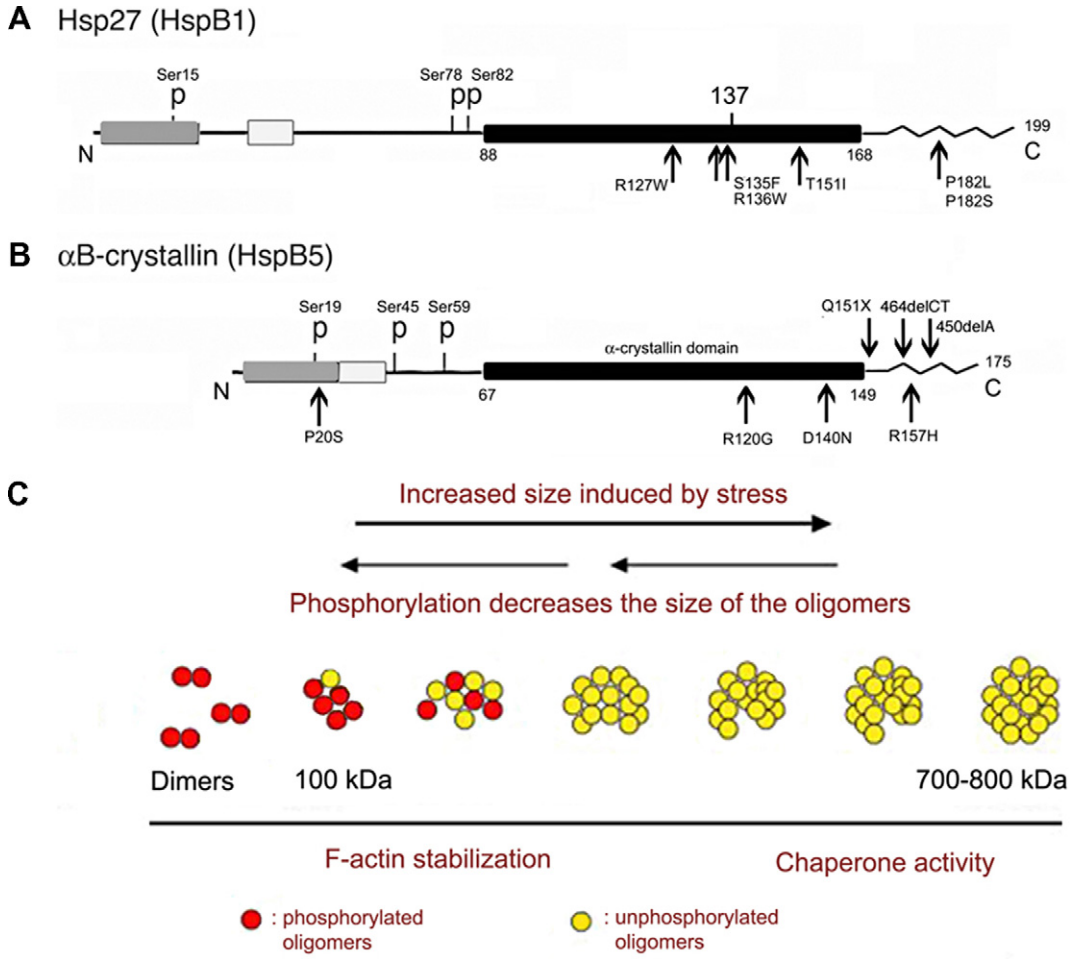

Fig. 1. Properties of human Hsp27 and αB-crystallin. (A) Organization of human Hsp27 and αB-crystallin protein sequences. Light box: conserved region; black box: alpha crystallin domain; gray box: WDPF domain; ΛΛΛΛΛ: flexible domain; P: phosphorylated serine residues. Amino acids are indicated. Position 137 in Hsp27 sequence corresponds to the only cysteine residue in the protein sequence. Its deletion abolishes dimer formation and knocks out Hsp27 protective activity. Positions of point mutations that are responsible of pathologies (see Table 2) are indicated by arrows. 464delCT: frame-shift mutant. The resulting mutant is modified from aa 155 and is truncated of 13 residues compared to wild type protein. 450delA: frame-shift mutant. The resulting mutant is modified from aa160 to aa184. This protein is larger than wild type polypeptide (175aa). (B) Biochemical properties of Hsp27. Stress favors the formation of large oligomers associated with unfolded polypeptides while phosphorylation does the reverse. The system is therefore in equilibrium. The formation of small oligomers may be required to bind unfolded proteins that are then stored at the level of the large oligomers. Phosphorylation may also favor the recycling of the large oligomers. Yellow circles indicate nonphosphorylated Hsp27 and red circle phosphorylated Hsp27. Large non-phosphorylated oligomers of sHsp (>300 kDa) have greater potentiality to protect the cell through their ability to display chaperone activity [6]. In contrast, small unphosphorylated oligomers of Hsp27 may act at the level of F-actin polymerization/depolymerization [32].

specific expression in the absence of stress which can be detected in the healthy adults as well as during the development of the organisms. In mammals, αB-crystallin is a major polypeptide of the eye lens where it is associated with the closely related αA-crystallin (HspB4) to form large hetero-oligomeric structures. In mice and rats, αB-crystallin is also constitutively expressed in tissues with high rates of oxidative metabolism, including, the heart, type I and type IIa skeletal muscle fibers, brain and oxidative regions of the kidney. Hsp27 tissue-specific expression resembles that of αB-crystallin. However, different levels of expression of these two proteins are often detected. The significance of the constitutive expression of these Hsps is probably linked to protection of the cells against stress or to a specific function in a particular tissue. This review summarizes the current knowledge about Hsp27 and αB-crystallin as well as the significance of the overexpression of these polypeptides in several pathological situations. Collectively, these observations lead to the conclusion that Hsp27 and αB-crystallin are major targets for the development of future therapeutic strategies against pathologies as diverse as neurodegenerative diseases, myopathies, asthma, cataracts and cancers.

Analysis of Hsp27 and αB-crystallin oligomers has revealed that these structures are in a dynamic equilibrium. It was then shown that the high molecular weight oligomeric structures formed by Hsp27 and αB-crystallin bear an ATP-independent chaperone activity and that phosphorylation induces modifications in oligomer size and chaperone-like activity [8]. For example, in heat shock treated cells which are prone to accumulate misfolded proteins, the large unphosphorylated oligomers of Hsp27 act as tanks that store misfolded polypeptides until they are either processed for refolding by ATP-dependent chaperones (i.e. Hsp70 and co-chaperones) [9] or degraded by the proteasome [10]. Recent studies of α B-crystallin have revealed that the β3 sequence of the α-crystallin domain (aa

Table 1
Multiple functions of αB-crystallin and/or Hsp27 and the corresponding interactions with (or functional modulation of) protein or peptide targets

Function(s)	Targets
Lens transparency and protection	αA-crystallin and other crystallin proteins
Heart protection	Titin, Hsp20
Cytoskeletal architecture and protection	F-actin Intermediate filament proteins (desmin, vimentin, GFAP, neurofilaments, filensin, phakinin, lamin) Microtubules and microtubule-associated proteins
Apoptosis resistance	Pro-caspase 3, cytochrome *c*, Smac/Diablo, Akt, DAXX, STAT3, Bcl-xs, Bax, P53
Ubiquitin–proteasome system	Fbx4, C8/α7 subunit of 20S proteasome, eIF4F and eIF4G complex, ubiquitin
Cell cycle regulation	Cyclin D1, p27kip1, P53
Redox homeostasis	Glutathione, G6PDH
Protein intracellular transport	Microtubule, SMN, neurofilaments
Stress signalling pathway	P38 cascade, I Kappa B kinase
Hormone signalling pathway	ERb (Estrogen cascade), hGMEB1 (glucocorticoïd hormones cascade)
Unknown nuclear function(s)	SMN, SC35
Unknown cytosolic function(s)	αB-crystallin, Hsp20, Hsp22, Hsp27
Pathological-related misfolded proteins	Desmin, GFAP, neurofilaments, ZASP, filamin C, myotiline, parkin, α-synuclein, prion protein, tau, β-amyloid, huntingtin, serpin, SOD, P150 dynactin, αA-crystallin, αB-crystallin, Hsp20, Hsp22, Hsp27
Virus	NS5A protein from Hepatitis C
Immune response	CD10, β2-microbulin
Unknown function in Sertoli cells	PASS 1
Golgi architecture	GM130

GFAP, glial fibrillary acidic protein; DAXX, death domain-associated protein 6; STAT3, signal transducer and activator of transcription 3; Fbx4, F-box only protein 4; eIF4F, eukaryotic translation initiation factor 4F; eIF4G, eukaryotic translation initiation factor 4G; G6PDH, glucose-6-phosphate dehydrogenase; SMN, survival motor neuron protein; SOD, superoxide dismutase; ER, estrogen receptor; SC35, splicing factor; hGMEB1, human glucocorticoid modulatory element-binding protein 1; NS5A, non-structural protein 5A; ZASP, LIM domain-binding protein 3; PASS 1, protein associated with small stress protein 1. GM130, golgi matrix-protein 130.

73–85) [11] may represent the interacting site with unfolded polypetide targets and the β3-β8-β9 surface of the alpha crystallin core domain may be an interface for complex assembly and chaperone activity [12]. Moreover, the N- and C-termini of human αB-crystallin appear important for the recognition, selection, and solubility of substrate proteins [13]. Hsp27 and αB-crystallin also share the ability to participate in the so-called ''protein triage'' that occurs in cells recovering from stress or committed to differentiate. Indeed, Hsp27 and αB-crystallin modulate the ubiquitin–proteasome pathway [14,15] (see Table 1) and are essential for proper disassembly–assembly of protein complexes to prevent undesirable interactions and aggregation [16,17]. In this respect, the lack of Hsp27 expression during early differentiation induces aberrant cell differentiation [16] or massive apoptosis [18–20]. Moreover, tissue-specific hetero-oligomeric structures of sHsps have been described [16] suggesting structurally independent sHsps chaperone complexes with distinct molecular targets [21]. Hence, these observations favor the hypothesis that highly modulable sHsp structural networks exist in the cell that rapidly react to cope with tissue-specific stress- or differentiation-induced protein damages and/or protein complexes reorganization.

An intriguing function of Hsp27 and αB-crystallin is the ability to increase the resistance of cells to oxidative injuries [22]. The phenomenon is not restricted to cell cultures and has been observed in whole animals [23]. Hsp27 and αB-crystallin expression correlates with decreased levels of reactive oxygen species (ROS) and nitric oxide ($NO^{\bullet}$) [6,24–26]. Consequently, in cells exposed to oxidative challenges, sHsps expression reduces lipid peroxidation, protein oxidation and F-actin architecture disruption [24–27]. These Hsps also uphold the mitochondrial membrane potential ($\Delta\Psi m$) level [24,28]; a phenomenon which provides the stressed cells with abundant ATP production that favors the activity of chaperones. The antioxidant activity of Hsp27 and αB-crystallin was found to depend on reduced glutathione [25]. The phenomenon probably depends on the upregulation of glucose-6-phosphate dehydrogenase (G6PDH) [23,24] (Table 1), the key enzyme that provides the reducing power of the cell by reducing NADP+ in NaDPH(H)+. In addition, recent results have shown that Hsp27 [29,30] or αB-crystallin (our unpublished information) expression decreases iron intracellular levels; a phenomenon which subsequently interferes with the formation, through iron-dependent Fenton reaction, of the potent macromolecules oxidizing hydroxyl radical (OH^{O}) [26]. As in the case of cells exposed to heat shock, the active form of Hsp27 appears associated with the large unphosphorylated oligomers of the protein [6,31].

Other functions have been assigned to Hsp27 and αB-crystallin that depend on the structural organization of these polypeptides. One example is the control of F-actin cytoskeletal integrity mediated by the small oligomers of Hsp27 [32,33]. This activity plays a crucial role in cells exposed to heat shock or oxidative stress because of the well-known ability of these stress to collapse F-actin cytoskeleton. Hsp27 was also found to regulate neutrophil chemotaxis and exocitosis through actin reorganization [34,35]. Moreover, a decrease in the level of expression of Hsp27 impairs growth and cytoskeletal organization [36]. These observations confirm that these polypeptides are major regulators of actin polymerization-depolymerization process. Moreover, Hsp27 and αB-crystallin also appear to bind and stabilize microtubules [1,37–39]. Taken together with the fact that αB-crystallin is a well known stabilizer of intermediate filaments [40,41], these observations enlighten the major role played by these Hsps in cytoskeletal architecture homeostasis.

Increased cellular resistance to several pro-apoptotic agents or conditions is observed in cells expressing high loads of Hsp27 [42–44]. On the opposite, inhibition of Hsp27 expression

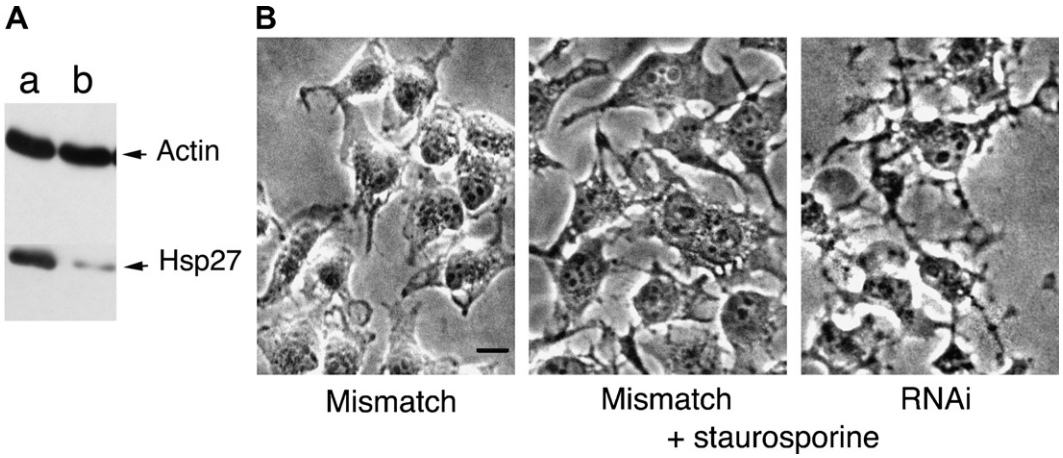

Fig. 2. Anti-apoptotic protective activity of constitutively expressed Hsp27 in HeLa cells. HeLa cells are human cancerous cells of the cervix that constitutively express high levels of Hsp27. These cells were transiently transfected with DNA vectors encoding either mismatch RNA (a) or RNAi Hsp27 sequences (b) (according to [104]). Two days after transfection, cells were either kept untreated or treated for 4 h with 0.2 μM of the kinase inhibitor and apoptosis inducer staurosporine. Hsp27 immunoblots (A) and phase contrast pictures (B) are presented. Note the drastic increase in cell death morphology induced by Hsp27 withdrawal. This leads to the conclusion that Hsp27 enhances the deleterious apoptotic resistance of these cancer cells. Bar: 15 μm.

sensitizes cells to apoptosis [45–48] (see Fig. 2). These phenomena result of the interaction of Hsp27 with several crucial apoptotic factors (see Table 1). For example, towards the intrinsic apoptotic pathway, Hsp27 acts upstream of mitochondria towards the signals that trigger the release of cytochrome *c* [45] or Smac/DIABLO [49] from mitochondria. In this respect, the ability of Hsp27 to protect F-actin network integrity [45] may play a crucial role. Hsp27 also acts down-stream of mitochondria at the level of cytochrome *c* and apoptosome [50]. The third site of action of this protein is at the level of pro-caspase 3 activation [51]. At the level of the Fas receptor pathway, Hsp27 was described to negatively interfere through an interaction with DAXX [52]. Hsp27 also binds and inhibits cellular factors involved in oncogenic signaling pathways, such as signal transducer and activator of transcription-3 (STAT3) [53]. This transcription factor is constitutively active in most tumors and controls the expression of key genes involved in cell transformation or apoptosis inhibition, such as those encoding Bcl-xL and survivin. An other important factor modulated by Hsp27 is Akt [54]. In vivo, Hsp27 anti-apoptotic property has been demonstrated [55,56]. Moreover, the transient expression of Hsp27 during cell differentiation is also related to a protection against apoptosis [18,43,57]. Concerning the structural organization of Hsp27 in cells exposed to apoptotic stimuli, the major information available today is that the large oligomers of Hsp27 inhibit in vitro caspase activation [58], hence suggesting a link with the chaperone activity of the protein.

Concerning αB-crystallin, its overexpression confers protection against a large panel of apoptotic stimuli while its silencing sensitizes cells to apoptosis [20,42,59,60]. Moreover, αB-crystallin negatively regulates apoptosis during myogenic differentiation [20]. Several steps in the apoptotic pathway are modulated by αB-crystallin. This protein has been shown to bind pro-apoptotic Bax, Bcl-x_S and P53 polypeptides (see Table 1) and to prevent their translocation to the mitochondria [61,62]. Downstream of mitochondria, αB-crystallin counteracts the activation of pro-caspase-3. Interestingly, phosphorylation of αB-crystallin at the level of serine 59 appears sufficient to provide maximal protection of cardiomyocytes against apoptosis [63].

2. Protein conformation and inflammation related diseases

In vivo, Hsp27 and αB-crystallin are abundantly produced in response to various types of stress in cardiac and skeletal muscles as well as in the brain. This suggests that, in these organs, these sHsps act as molecular chaperones suppressing the aggregation of specific client polypeptides. For example, transgenic mice overexpressing Hsp27 are strongly protected against myocardial infarction and cerebral ischemia [56,64]. Moreover, αB-crystallin and Hsp27 are often upregulated and accumulate into inclusion bodies in many protein conformation diseases. For instance, αB-crystallin and/or Hsp27 accumulate in Rosenthal fibers of Alexander disease, cortical Lewy bodies, Alzheimer disease plaques, neurofibrillary tangles as well as in synuclein deposit associated to Parkinson disease or myopathy-associated inclusion body [65]. The exact reason for the frequent association of Hsp27 and/or αB-crystallin with these structures is probably linked to the chaperone activity of these sHsps. Indeed, molecular chaperones are known to provide a first line of defence against misfolded, aggregation-prone proteins probably because of their ability to modulate the earliest aberrant protein interactions that trigger pathogenic cascades. For example, it has been reported that sHsps protect against alpha-synuclein [66], huntingtin [26,67,68], amyloid and desmin mutants induced aggregation and/or toxicity.

Other studies have reported that αB-crystallin is present in reactive glia in Creutzfeldt-Jakob disease and a high prevalence of anti-alpha-crystallin antibodies has been described in multiple sclerosis which correlates with severity and activity of the disease. Upregulation of Hsp27 has also been observed in a transgenic model of ALS. The importance of Hsp27 in neuropathologies was further confirmed by the discovery of

Table 2
Mutations in αB-crystallin and Hsp27 and the corresponding pathologies

sHsps	Mutations	Associated pathologies	Ref.
αB-crystallin	R120G	Myofibrillar myopathy, cardiomyopathy, cataract	(1)
	Q151X	Myofibrillar myopathy	(2)
	464delCT	Myofibrillar myopathy	(2)
	R157H	Cardiomyopathy	(3)
	P20S	Cataract	(4)
	D140N	Cataract	(5)
	450delA	Cataract	(6)
Hsp27	R127W	Distal hereditary motor neuropathy	(7)
		Charcot-Marie-Tooth type 2F	(9)
	S135F	Distal hereditary motor neuropathy Charcot-Marie-Tooth type 2F	(7)
	R136W	Charcot-Marie-Tooth type 2F	(7)
	T151I	Distal hereditary motor neuropathy	(7)
	P182L	Distal hereditary motor neuropathy	(7)
	P182S	Distal hereditary motor neuropathy	(10)

Refs: (1) [71]. (2) [72]. (3) [78]. (4) [77] (5) Berry, V. et al. (2001) Am. J. Hum. Genet. 69, 1141–5. (6) Liu, Y. et al. (2006) Invest. Ophthalmol. Vis. Sci. 47, 1069–1075. (7) Evgrafov, O.V. et al. (2004) Nat. Genet. 36, 602–606. (8) Tang, B. et al. (2005) Arch. Neurol. 62, 1201–1207. (9) Liu, X.M. et al. (2005) Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 22, 510–513. (10) Kijima, K., Numakura, C., Goto, T., Takahashi, T., Otagiri, T., Umetsu, K. and Hayasaka, K. (2005) J. Hum. Genet. 50, 473–476.

human mutations in the Hsp27 encoding gene in families associated with inherited peripheral neuropathies [69] and axonal Charcot-Marie-Tooth disease [70] (see Table 2). These motor neuropathies are caused by premature axonal loss, neuronal death and subsequent degeneration. Moreover, the mutations are associated with a decreased ability of Hsp27 to promote neuronal survival compared to the wild type protein. Taken together, these studies suggest that, in animal models of human diseases, Hsp27 and αB-crystallin are potent suppressors of neurodegeneration.

Other protein conformation diseases associated to Hsp27 and/or αB-crystallin expression are myopathies and alcoholic liver diseases characterized by the presence of Mallory bodies. Concerning the myopathies, recent studies have revealed the importance of αB-crystallin towards desmin network (see Table 2). Indeed, one major target of the chaperone activity associated to αB-crystallin appears to be type III intermediate filaments [40,41]. The discovery in 1998 of a missense mutation in αB-crystallin gene, changing arginine 120 to glycine (R120G), responsive of a myofibrillar myopathy associated with cardiomyopathy and cataract [71], confirmed the importance of αB-crystallin in these diseases. Recently, two novel mutations leading to myofibrillar myopathies (Q151X and 464delCT) have been identified in the terminal part of the αB-crystallin coding sequence [72]. At the exception of the report describing the identification of these new mutants, the published studies on αB-crystallinopathies concern the R120G mutant. It is now accepted that αB-crystallinopathies result from the misfolding and progressive aggregation of mutated αB-crystallin to which subsequently associate desmin filaments to form αB-crystallin/desmin/amyloid positive aggresomes [73,74]. These aggregates can by themselves be toxic, inhibiting the ubiquitin–proteasomal system of protein degradation [75] and causing deficits in mitochondrial function [76]. αB-crystallinopathies are a special case of protein conformation disease in which the destabilizing mutations at the origin of the disorder occurs in a molecular chaperone which is itself potentially involved in the protein quality control of the cell. Three mutations in αB-crystallin gene (P20S, 464delCT and D140N) are also responsive for dominant cataract [77] and two mutations (R157H and G154S) for cardiomyopathy [78]. At the biochemical level, mutations in αB-crystallin have been found to modify the properties of αB-crystallin such as its oligomerization and in vitro chaperone-like activity [79] and to increase its affinity to desmin [80]. Moreover, the ability of αB-crystallin to interact with members of the apoptotic cascade, cytoskeletal polypeptides or with the other sHsps may also be modified.

Hsp27 and αB-crystallin are also involved in inflammation diseases. For example, these proteins interferes with TNFα signaling pathway through their ability to protect against oxidative stress [81] and through modulation of TAK-1 activity [82]. An other example is given by the absence of colonic inflammation seen in the majority of individuals infected with the parasite *Entamoeba histolytica* which is related to the ability of Hsp27 to suppress NF-κB activation through an interaction with IKK-α and IKK-β [83]. Moreover, recent reports have shown that Hsp27 is needed for the activation by interleukin (IL)-1 of TAK1 and downstream signalling by p38 MAPK, JNK and their activators (MKK-3, -4, -6, -7) and IKKβ [82]. These observations suggest crucial roles of Hsp27 and αB-crystallin in the control of inflammatory processes. Among pathologies where the anti-oxidative potential of Hsp27 is crucial, one can cite airway inflammation associated with asthma which is characterized by the damage of the bronchial epithelium. In this respect, we have observed that an increased Hsp27 expression in the epithelium of asthmatic subjects generates a protection against the oxidative stress induced by the chronic inflammatory state of this tissue (see Fig. 3) [84].

3. Cancer

High levels of Hsp27 constitutive expression have been detected in several cancer cells, particularly those of carcinoma origin [85,86]. Recently, the number of reports dealing with Hsp27 in cancer pathologies has grown exponentially. In addition to its presence in breast, ovary and colon cancers, Hsp27 has recently been detected in liver, kidney, lung (non-small

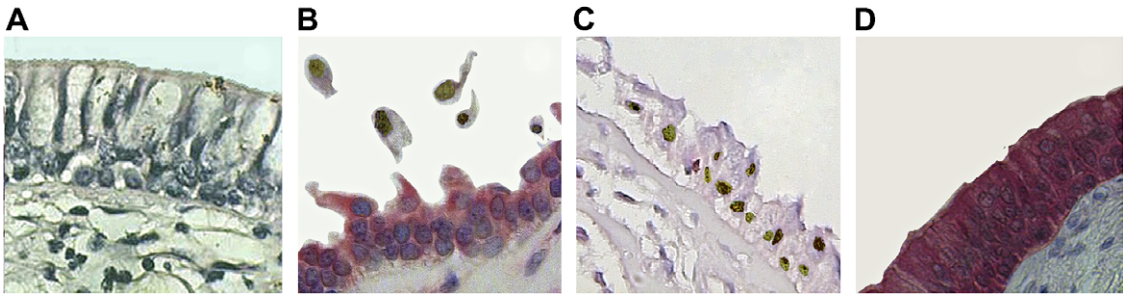

Fig. 3. Beneficial protective effect of Hsp27 expression in bronchial epithelial cells from asthmatic patients. Hsp27 expression and TUNEL immunoreactivity in bronchial epithelial cells of a normal (A) subject and an asthmatic (B–D) patient. Cells that express Hsp27 show a red immunostaining as the result of the Hsp27 immunoreactivity. TUNEL-positive cells are characterized by a brown staining of the nuclei. (A) Area of intact epithelium of a bronchial biopsy taken from a normal subject showing no immunoreactivity for Hsp27 and for TUNEL. (B) Area of damaged epithelium of a bronchial biopsy taken from an asthmatic subject with desquamated epithelial cells which are not immunoreactive for Hsp27 but show a positivity for the TUNEL technique. (C) Wide area of fragile epithelium not immunoreactive for Hsp27 showing a nuclear TUNEL staining of many bronchial epithelial cells. (D) Area of intact epithelium showing a strong immunostaining for Hsp27, and a complete lack of nuclear staining due to the TUNEL technique. These observations support the beneficial role of Hsp27 in asthma. Indeed, desquamative cells are apoptotic and devoid of Hsp27 while cells that express Hsp27 are not apoptotic and do not desquamate. See [84] for further informations.

cells) and prostate cancers. Moreover, experimental approaches performed in rodents have enlightened the tumorigenic potential of Hsp27 expression [87]. Hence, Hsp27 is supposed to increase the ability of some cancer cells to resist to and evade from the apoptotic processes mediated by the immune system. The large oligomers which bear the chaperone-like activity are also responsible for the tumorigenic activity of Hsp27 [58]. Concerning this issue, it cannot be excluded that Hsp27 large oligomers may act as Hsp90 and bind specific client proteins that participate in the tumorigenic and metastatic processes.

αB-Crystallin constitutive expression has been detected in gliomas, prostate cancer, oral squamous cell carcinomas, renal cell carcinomas, head and neck cancer. A normal high level of αB-crystallin has also been detected in basal-like breast carcinomas and preinvasive ductal carcinoma that correlated with poor clinical outcome of the patients. Recently, a pathological role of αB-crystallin has again been reported in breast cancer diseases, hence suggesting that this protein acts as an oncoprotein [88]. Neoplastic changes and invasive properties of breast cells are inhibited by phosphorylation of αB-crystallin [89]. Indeed, serine 59 phosphorylation reduces the oligomerization and anti-apopototic activities of αB-crystallin [90,91]. It can therefore be concluded that, as for Hsp27, the large oligomers of αB-crystallin may contribute to the aggressive behavior of cancer cells. However, the differential expression of αB-crystallin and Hsp27 reported in anaplastic thyroid carcinomas and in brain cancer suggests different involvement of these sHsps in these pathologies [92,93].

The expression of Hsp27 and αB-crystallin is also associated to other problems in cancer biology. First, high levels of Hsp27 are observed in metastatic tissues compared to non metastatic tissues suggesting that this protein plays a key role in metastasis formation [94]. αB-crystallin expression is correlated with lymph node involvement in breast carcinomas resulting in a shorter survival. Second, Hsp27 and αB-crystallin expression is associated with cellular resistance to cytostatic anticancerous drugs used in the clinic [95,96]. In addition, some of these drugs, particularly cisplatin [97], vincristine and colchicine [98] enhance Hsp27 and/or αB-crystallin expression. Collectively, these phenomena impair the efficiency of the clinical treatments using chemotherapeutic agents.

4. Hsp27 and αB-crystallin as therapeutic targets?

Hsp27 and αB-crystallin are potent protective factors of cells in which the disease-causing proteins are prone to aggregate and form large inclusions. In spite of the presence of Hsp27 and/or αB-crystallin, these diseases usually result in excessive cell death (Fig. 4). One therapeutic option could be the enhanced expression of the corresponding wild type protein. Unfortunately, such an approach is not feasible nowadays. Similarly, this approach can not be used to treat pathologies caused by Hsps mutations. Another approach to the development of therapeutic intervention for these diseases has been to identify chemical compounds that reduce the size or number of inclusions. However, recent results suggest that inclusion formation may in fact be beneficial to the cell to get rid of the mutated protein through autophagy [99]. However, inclusion formation or aggregation of metal-binding proteins (such as α-synuclein, Alzheimer β-amyloid peptide, PrP106-126 prion or polyQ mutants of Huntingtin) is a risky process since it can generate deleterious oxidative stress [67,100]. Hence, using available cellular models, studies will have to be performed to identify compounds that promote oxidative stress free inclusion formation as a therapeutic approach for neurodegenerative diseases caused by protein misfolding [101]. These studies will have to test whether compounds (still to be discovered, such as RNA/peptide aptamers or chemical chaperones, see below) that modulate Hsp27 and/or αB-crystallin functions are active towards the deleterious damages induced by these diseases.

In the myopathy and cataract research field, it is reasonable to assume that prevention of the formation of aggregates induced by αB-crystallin myopathy- and cataract-associated mutants may be an efficient strategy to inhibit the development of the disease (Fig. 4). For example, it is well known that the cessation of the expression of αB-crystallin R120G mutant in symptomatic mice improved cardiac function and rescued these animals from premature death [73]. Towards these pathologies, specific peptide/RNA aptamers or chemical chaperones that interfere with the mechanism leading to the aggregation of the mutated αB-crystallin but not with the wild type protein functionality should be researched and tested. A similar approach can be proposed towards the pathologies induced by mutations of Hsp27.

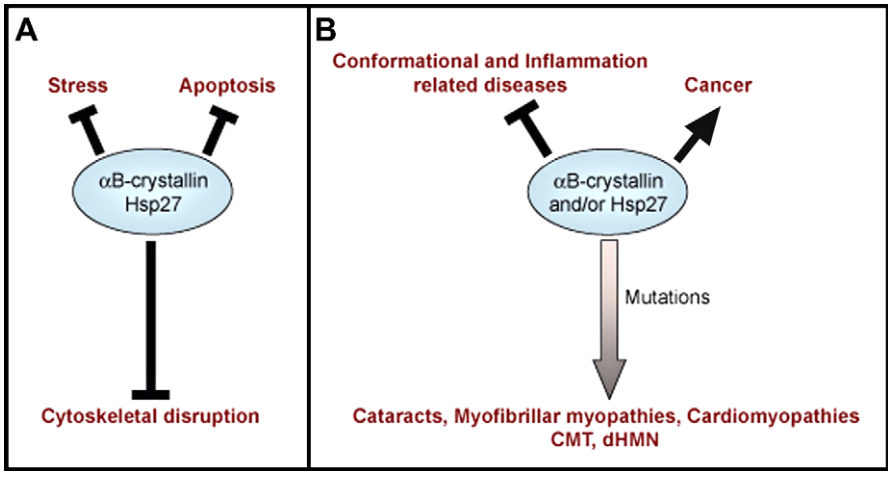

Fig. 4. Role of Hsp27 and αB-crystallin in normal and pathological cells. (A) In normal cells, Hsp27 and/or αB-crystallin participate in cytoskeleton, redox state and protein folding homeostasis. These proteins are also involved in the protection of cells in case of stress. In this respect, these sHsps interfere with spontaneous or induced apoptosis. (B) In pathological cells, Hsp27 and/or αB-crystallin have beneficial effects towards protein conformation and inflammation related diseases. In contrast, these proteins can have pernicious effects through their ability to protect cancer cells against the immune system- or drug-mediated death. Mutations in Hsp27 and/or αB-crystallin are responsive of the development of pathologies such as, cataracts, myofibrillars myopathies, cardiomyopathies, Charcot-Marie-Tooth (CMT) disease and motor-neuronal neuropathies, such as Distal Hereditary motor neuronopathy (dHMN).

Towards inflammation and asthma, compounds that stimulate the anti-oxidative activity of Hsp27 and αB-crystallin should be actively researched. Once again specific peptides or RNA aptamers or drugs that maintain these proteins in the form of large oligomers may stimulate their chaperone and anti-oxidative properties.

In the cancer field, Hsp27 and αB-crystallin have negative activities and should be either eliminated or their activity impaired (Fig. 4). Indeed, up until today, no report has described a positive role of these proteins in cancer cells, such as better tumor antigen-recognition at the cell surface as already shown in the case of Hsp70 [102,103]. Hence, experiments have been performed using anti-sense or nucleotide-based therapies with aim to inhibit Hsp27 and αB-crystallin expression. This approach sensitizes cancer cells to apoptotic inducers [45] and anticancer drugs and reduces the tumorigenic potential of bladder and prostate cancer cells [46,48]. The decrease in tumors aggressivity mediated by second generation of RNAi molecules, such as OGX 437 (Oncogenex Inc.), appears to be linked to loss of the anti-apoptotic protection mediated by Hsp27 [48]. Moreover, the degradation of putative and still unknown tumorigenic and/or metastatic client proteins that may bind Hsp27 should also be considered. As mentioned above, peptide/RNA aptamers or chemical chaperones that bind specific structural organizations of Hsp27 or αB-crystallin could be an alternative approach to reduce the tumorigenic and metastatic activities of these proteins. Similarly aptamers or drugs that modulate the anti-oxidant potential of these proteins (see above) may prove useful to block Hsp27 ability to counteract the killing efficiency of redox state dependent anti-cancer therapeutic drugs, such as 17AAG, or physical challenges such as X-rays irradiation.

5. Conclusions and perspectives

The number of reports that describe the importance of Hsp27 and αB-crystallin in pathologies is increasing exponentially and the need of drugs that modulate the activity of these chaperones is rising fast. The discovery of drugs will be a challenge since the tri-dimensional structures of human Hsp27 and αB-crystallin is still not known because of the difficulty to obtain stable crystals of these oligomeric proteins for X-ray analysis. In spite of these considerations, an astonishing array of strategies to either stimulate the beneficial properties or to affect the pathological roles played by these proteins is fast emerging. For example, the discovery of specific peptides that recognize the oligomeric forms of Hsp27 or αB-crystallin may prove useful to determine the structure of peptido-mimetic compounds leading to the emergence of chemical drugs designated to modulate specific activities of Hsp27 and αB-crystallin and/or which can correct and mask specific mutations in these chaperones. Hence, it is likely that in a near future drugs will be available for clinical trials. In addition, the intense work which is actually performed towards the eight other members of the human small stress family of proteins and the fact that most sHsps interact with each other and form homo- and hetero-oligomeric complexes may lead to the emergence of other interesting therapeutic targets and strategies.

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