Minireview

Hsp90 isoforms: functions, expression and clinical importance

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Received 9 January 2004; revised 24 February 2004; accepted 25 February 2004

First published online 8 March 2004

Edited by Felix Wieland

Abstract The 90 kDa heat shock protein, Hsp90, is a main functional component of an important cytoplasmic chaperone complex, and it is involved in various cellular processes, such as cell proliferation, differentiation and apoptosis. Identification of Hsp90 as a molecular target of various anticancer drugs highlighted its importance from the clinical point of view. Here we summarize the current knowledge on various Hsp90 isoforms regarding their genomic location, molecular evolution, functional differences, differential induction after various environmental stresses and in pathological conditions as well as the growing importance of discriminating between Hsp90 isoforms in clinical practice.

Key words: Anticancer drug; Chaperone; Heat shock protein; Molecular evolution; Stress; Tumor therapy

1. Introduction: Hsp90, an abundant chaperone

The 90 kDa heat shock protein, Hsp90, is one of the most abundant proteins in eukaryotic cells, comprising 1–2% of cellular proteins under non-stress conditions. Its contribution to various cellular processes including signal transduction, protein folding, protein degradation and morphological evolution has been extensively studied [1–5]. The full functional activity of Hsp90 is gained in concert with other co-chaperones, playing an important role in the folding of newly synthesized proteins and stabilization and refolding of denatured proteins after stress. Apart from its co-chaperones, Hsp90 binds to an array of client proteins, where the co-chaperone requirement varies and depends on the actual client. There is a growing list of Hsp90 client proteins, now including several hundred proteins. Interestingly, most of the clients include molecules involved in signal transduction [5].

2. Hsp90 genes and isoforms

There are two major cytoplasmic isoforms of Hsp90, Hsp90α (inducible form/major form) and Hsp90β (constitutive form/minor form) [1]. A recent report added another isoform to the Hsp90 family, Hsp90N, which is associated with cellular transformation [6]. Additional Hsp90 analogues include Grp94 in the endoplasmic reticulum and Hsp75/TRAP1 in the mitochondrial matrix [1]. Hsp75 possesses a unique LxCxE motif [7], which is absent in all other Hsp90 family members, and depends on stress kinases for its transcriptional activation [8]. We have mapped *hsp90α* and *hsp90β* to the human genome, and our search identified cDNA sequences in chromosomes 1, 3, 4, 11 and 14 for *hsp90α* and in chromosomes 4, 6, 10, 13, 14 and 15 for *hsp90β* showing several novel copies in the genome and raising the possibility of several pseudogenes of human *hsp90*. The genomic locations of human *hsp90α* at 14q32–33, *hsp90β* at 6p21 and *hsp75* at 16p13.3 are recognized as functional (http://www.infobiogen.fr [9,10]). Mammalian *hsp90* genes, in contrast to most eukaryotic *hsp* genes, are composed of several introns and exons (Fig. 1) [1]. The nucleotide sequences of *hsp90α* and *hsp90β* have much less similarity compared to the sequences of their protein products, particularly in their 5′ and 3′ non-coding regions, the introns, and the regulatory 5′ flanking sequences [11,12]. In contrast, the *hsp90N* gene shares a high similarity with that of *hsp90α* [6]. Hsp90 isoforms have five highly conserved regions, called 'signature sequences', of which three are in the N-terminal domain (Fig. 2; amino acids 38–59, 106–114, 130–145 of human Hsp90α) and two in the middle domain (amino acids 360–370 and 387–401) [12].

3. Molecular evolution of Hsp90 isoforms

Studies on the phylogenetic distribution of *hsp90* have concentrated on the cytoplasmic isoforms [12,13]. The mammalian *hsp90* isoforms α and β arose by gene duplication roughly 500 million years ago [12]. There is a relatively high conservation within these isoforms, and it was proposed that not only mammalian Hsp90 acquired a novel chaperone activity, but the α and β isoforms may also display different chaperone activities [14]. Available data suggest that the endoplasmic reticulum *hsp90* homologue originated via a paralogous gene duplication very early in eukaryotic evolution [12]. In contrast, the additional *hsp90* isoform *hsp90N* represents a very recent gene rearrangement of *hsp90α* [6].

4. Biochemical differences between Hsp90 isoforms

Since the biochemical separation of Hsp90 isoforms is rather difficult, most studies have been carried out with a mixture of the α and β Hsp90 isoforms. An important differ-

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0014-5793/04/$30.00
doi:10.1016/S0014-5793(04)00229-7

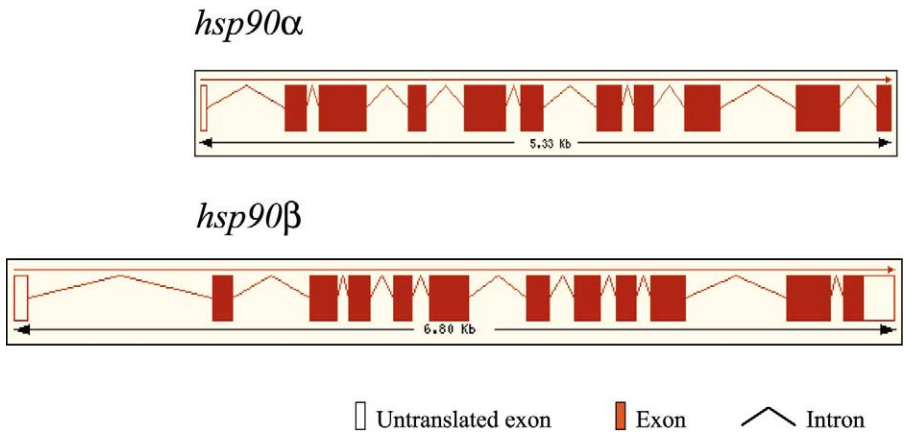

Fig. 1. A schematic view of the intron/exon structure of human *hsp90* isoforms. Data are from www.ensembl.org.

ence is that the α form readily dimerizes, whereas the β form does so with much less efficiency. Hsp90 is mainly a constitutive dimer (αα or ββ), however, monomers (α or β), heterodimers (αβ) and higher oligomers of both isoforms also exist. The dimerization potential resides mainly at the carboxy-terminal 190 amino acids of Hsp90 (Fig. 2) [1,6]. In addition, there are certain areas in the amino acid sequences that differ between the α and β isoforms raising the possibility of more isoform-specific functions, such as differential binding to client proteins. Though Hsp90N shares high sequence homology with the other two Hsp90 isoforms, it lacks the 25 kDa N-terminal domain, a highly conserved ATP binding site of Hsp90 [6]. The mitochondrial isoform Hsp75 is similar to the bacterial Hsp90 homologue, Hsp75, where the highly charged hinge region of the N-terminal section is missing [1,7].

5. Functional differences between Hsp90 isoforms

As expected, the isoform specificity is not restricted to the biochemical level but extends to the functional role of Hsp90 in cell differentiation and development. Thus a regulatory role of Hsp90α in muscle cell differentiation of zebrafish has been reported [15]. In contrast, overexpression of Hsp90α inhibited cellular differentiation of embryonal carcinoma cells to trophoectoderm [16]. Reduction of Hsp90α by disruption of heat shock factor (HSF) genes caused instability of the cyclin-dependent kinase cdc2 against thermal stress [17]. Hsp90β has been shown to play a major role in trophoblast differentiation, and Hsp90β-deficient homozygous mice with normal expression of Hsp90α failed to differentiate to form placental labyrinths [18]. Hsp90β overexpression is observed throughout the germ cell lineage from very early stages of development to adult oocytes and spermatocytes [19]. Later studies confirmed these observations in various models and different stages of development, and suggested that Hsp90β is required for early embryonic development [20–22]. Thus, there are multiple differences between Hsp90 isoforms in cell differentiation and embryonic development in various organisms.

There is both in vivo and in vitro evidence that Hsp90 – along with other Hsps – directly binds to tubulin and actin, the major components of the eukaryotic cytoskeleton [1]. Isoform-specific cytoskeletal interactions have been reported by Cambiazo et al. [23] suggesting that Hsp90β is the major microtubule-interacting protein, compared to Hsp90α.

Hsp90 is involved in cell survival and the various pathways leading to cell death, such as apoptosis or necrosis [24]. Our current knowledge of Hsp90 isoforms suggests that only the α isoform of Hsp90 is involved in nicotine-induced apoptosis as seen by DNA fragmentation and caspase-3 activation [25].

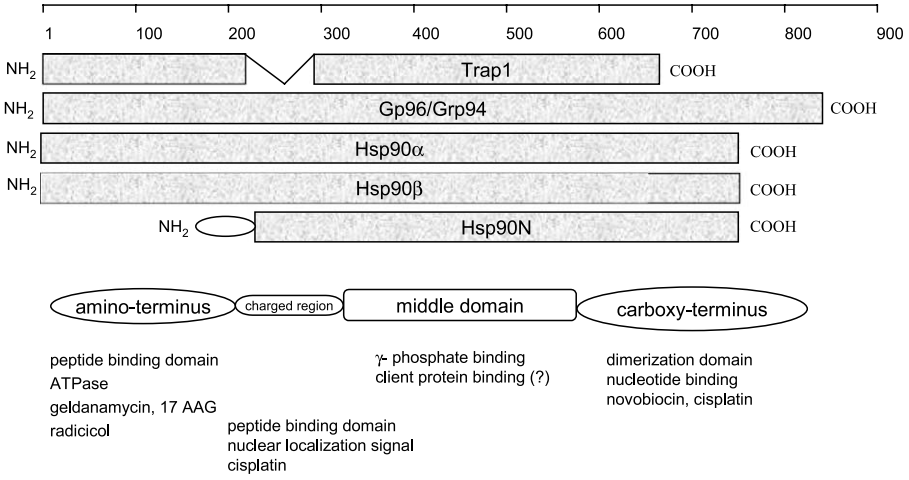

Fig. 2. Schematic representation of various Hsp90 isoforms. The numbering 1 through 900 refers to the amino acid sequence. The functional significance of the domains and the approximate position of the specific drug binding sites are listed.

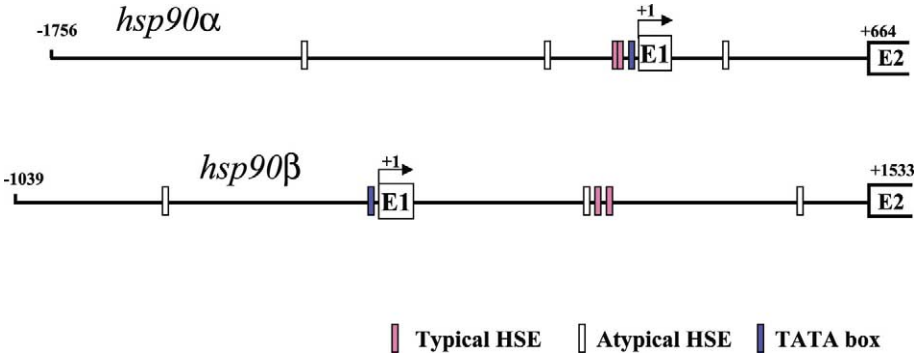

Fig. 3. Schematic representation of HSEs in the promoter region of human *hsp90α* and *hsp90β* genes. E1, exon 1; E2, exon 2.

6. Differences between the expression mechanisms of Hsp90 isoforms

The transcription of the *hsp90α* gene is tightly regulated by the 5′ upstream promoter sequences containing several heat shock elements (HSEs), which are involved in the inducible gene expression of *hsp90α* [9,26]. Quite interestingly, although Hsp90β is not regarded as a highly inducible family member of the 90 kDa Hsps [27], the typical HSEs in the first intron of the human *hsp90β* gene play a critical role as heat shock-dependent enhancers of transcription (Fig. 3). Initiation from within the first intron is important for the high constitutive expression as well as for the highly efficient heat inducibility of the *hsp90β* gene. HSF1 binds to a heat shock-specific enhancer composed of two typical HSEs in the first intron of the *hsp90β* gene, which efficiently enhances heat shock-induced transcription initiated from 3′ downstream sequences within the intron [28]. As a unique feature compared to intron-less *hsp* genes, both *hsp90α* and *hsp90β* regulate *hsp90* mRNA splicing after a severe stress in *Drosophila* [29]. The similarity of regulatory sequences for Hsp90 isoforms in different mammalian species is usually higher than that of the two Hsp90 isoform genes in one animal. For example, the 600 bp sequence in the proximal region flanking the mouse *hsp84* and that of human *hsp90β* gene are ~85% conserved [30], while those of the human *hsp90α* [26] and *hsp90β* genes [31] are completely different. In addition to the α and β isoforms of Hsp90, Hsp90N has been proposed to have differential promoter utilization and/or splicing [6].

Beyond the typical HSF/HSE-activating pathway for heat shock genes, we have recently found that the human *hsp90β* gene is specifically enhanced by the protein kinase Cε isoform in the heat shock response [32]. Additionally, Stat1, of the protein family of signal transducers and activators of transcription, plays a complex role in the expression of the heat shock-sensitive version, the *hsp90α* gene. Phosphorylated $Stat1^{Tyr701}$ appears after heat shock and restrains the overexpression of the *hsp90α* gene in Jurkat cells. The restrained *hsp90α* gene expression is released with the overexpression of a $Stat1^{Y701F}$ mutant or by insertion of a GAS (γ-interferon-activated sequence) element to nucleotides −1612/−1604 relative to the transcription start site of the *hsp90α* gene. Ectopic wild-type Stat1 represses the heat shock-induced expression of the *hsp90α* gene, but not that of its constitutive counterpart, *hsp90β* (X.S. Chen, J.S. Wang, X.Y. Li, F.Y. Heng, X.K. Cheng, N.H. Wu and Y.F. Shen, submitted for publication). It is thus suggested that the expression levels of the two versions of human *hsp90* genes may be accurately coordinated in vivo to accommodate the requirement of individual cellular functions in heat shock response.

7. Differential induction of Hsp90 isoforms

Hsp90α expression is lower compared to Hsp90β in most cells. *hsp90α* is highly inducible, and its selective induction in nicotine-induced apoptosis was cytoprotective [25]. An unusual, high expression of *hsp90α* was shown to be associated with tumor progression [33], enhanced cell cycle regulation [34], and growth factor-mediated signaling via tyrosine kinases [35]. In contrast to *hsp90α*, *hsp90β* expression is thought to be constitutive. However, *hsp90β* can also be induced. Hsp90β is probably involved in long-term cellular adaptation. In support of this, Hsp90β expression was shown to be associated with the development of drug resistance [36]. Due to its generally higher levels than Hsp90α, Hsp90β is the major form of Hsp90 involved in normal cellular functions, such as maintenance of the cytoarchitecture [37], differentiation [38], cytoprotection [24]. For more details of differential induction/expression of *hsp90* isoforms in response to various extracellular signals and stress events see the comprehensive review by Csermely et al. [1].

8. Hsp90 isoforms and clinical practice: isoform expression in tumors

There are a few reports that Hsp90 expression is associated with many types of tumors; however, Ogata et al. [39] showed that acute and chronic pancreatic tumors have enhanced Hsp90α expression, whereas benign tumors do not. In addition, overexpression of Hsp90α is associated with a poor prognosis of breast cancer [40], pancreatic carcinoma [41], and human leukemia [33]. In patients with systemic lupus erythematosus, the disease is associated with increased Hsp90β transcription [42]. Hsp90β expression has been shown to be associated with chronic tumors [39]. Hsp90β expression is implicated in multidrug resistance through its interaction with the P-glycoprotein, a key component of the development of multidrug resistance [36]. Though basal level expression of Hsp90N is observed in normal rat fibroblasts, overexpression of this protein results in cellular transformation through augmenting Raf/Ras activity [6].

9. Hsp90 isoforms and clinical practice: inhibition of Hsp90 isoforms

Hsp90 inhibition provides a recently developed, important pharmacological platform for anticancer therapy. With the inhibition of this pleiotropic chaperone, many survival and signaling pathways can be inhibited simultaneously. The inhibition pattern of a geldanamycin analogue in clinical trials shows a preference for cancer cells due to the higher-affinity binding to the cancer-specific, complexed form of Hsp90 [43]. Most of the Hsp90-related antitumor drugs, like geldanamycin analogues, radicicol and the purin scaffold inhibitors, are aimed to the N-terminal ATP binding domain (Fig. 2) [43–47]. The nucleotide binding pocket is fairly conserved in Hsp90α and Hsp90β, thus we cannot expect a major difference in the inhibition of these isoforms by the available Hsp90 inhibitors.

However, due to the lack of the N-terminal ATP domain in Hsp90N, these drugs are ineffective for the inhibition of the chaperone function of Hsp90N. Despite the missing N-terminal domain this Hsp90 isoform is still a chaperone [6], which is in agreement with the proposed chaperone activity of the highly conserved C-terminal domain of both Hsp90 isoforms being independent of that associated with the N-terminus [1]. Hence, it is important to design specific inhibitors for both the N-terminus and C-terminus of Hsp90. The recent finding of a C-terminal nucleotide binding site (Fig. 2) [48–50] and the characterization of its differential nucleotide binding specificity [51] might help to accomplish this task.

10. Conclusions and perspectives

The current understanding of Hsp90 as a molecular chaperone explains well its role in many cellular functions both in normal and in pathophysiological conditions. Hsp90 chaperone function alone, however, has failed to rationalize the isoform-specific function of this heat shock protein so far. In the cellular context Hsp90α emerges as a fast-response, cytoprotective isoform, while Hsp90β seems to be associated with long-term cellular adaptation and facilitated cellular evolution (Table 1). The first report on the isoform-specific variation of Hsp90 polymorphism in humans opens a wide arena for pharmacologists to design more specific inhibitors of Hsp90 for antitumor therapies [52]. Isoform-dependent Hsp90 polymorphism warns us further that the isoform specificity of Hsp90 functions needs much more study in the future. From this knowledge even more focused pharmacological intervention, like isoform-specific Hsp90 inhibitors, may also be born.

Table 1
Differences in the function and expression of Hsp90 isoforms

Isoform	Specific function	Major expression status
Hsp90α	growth promotion cell cycle regulation stress-induced cytoprotection	induced
Hsp90β	early embryonic development germ cell maturation cytoskeletal stabilization cellular transformation signal transduction long-term cell adaptation	constitutive
Hsp90N	cellular transformation	constitutive
Hsp75/TRAP1	cell cycle regulation	constitutive

Most of the differential functions are characteristic of both Hsp90α and Hsp90β, but they are mostly associated with the isoform mentioned. Cell proliferation and differentiation are regulated by both Hsp90α and Hsp90β.

Acknowledgements: Work in the authors' laboratory is supported by research grants from the EU 6th Framework program (FP6506850), the Hungarian Science Foundation (OTKA-T37357), the Hungarian Ministry of Social Welfare (ETT-32/03), the International Centre for Genetic Engineering and Biotechnology (ICGEB, CRP/HUN 99-02), the Natural Science Foundation of China (#39930050 and #3002-8011) and by a Chinese–Hungarian collaborative grant (CHN-12/03) and MS-1 from the Chinese Ministry of Science and Technology. A.S.S. is the recipient of a National Overseas Scholarship from the Ministry of Social Justice and Empowerment, Government of India.

References

[1] Csermely, P., Schnaider, T., Soti, C., Prohaszka, Z. and Nardai, G. (1998) Pharmacol. Ther. 79, 129–168.
[2] Pearl, L.H. and Prodromou, C. (2001) Adv. Protein Chem. 59, 157–186.
[3] Richter, K. and Buchner, J. (2001) J. Cell Physiol. 188, 281–290.
[4] Picard, D. (2002) Cell. Mol. Life Sci. 59, 1640–1648.
[5] Pratt, W.B. and Toft, D.O. (2003) Exp. Biol. Med. 228, 111–133.
[6] Grammatikakis, N., Vultur, A., Ramana, C.V., Siganou, A., Schweinfest, C.W., Watson, D.K. and Raptis, L. (2002) J. Biol. Chem. 277, 8312–8320.
[7] Felts, S.J., Owen, B.A., Nguyen, P., Trepel, J., Donner, D.B. and Toft, D.O. (2000) J. Biol. Chem. 275, 3305–3312.
[8] Kim, S.H., Kim, D., Jung, G.S., Um, J.H., Chung, B.S. and Kang, C.D. (1999) Biochem. Biophys. Res. Commun. 262, 516–522.
[9] Durkin, A.S., Maglott, D.R., Vamvakopoulos, N.C., Zoghbi, H.Y. and Nierman, W.C. (1993) Genomics 18, 452–454.
[10] Ozawa, K., Murakami, Y., Eki, T., Soeda, E. and Yokoyama, K. (1992) Genomics 12, 214–220.
[11] Hickey, E., Brandon, S.E., Smale, G., Lloyd, D. and Weber, L.A. (1989) Mol. Cell. Biol. 9, 2615–2626.
[12] Gupta, R.S. (1995) Mol. Biol. Evol. 12, 1063–1073.
[13] Stechmann, A. and Cavalier-Smith, T. (2003) J. Mol. Evol. 57, 408–419.
[14] Pepin, K., Momose, F., Ishida, N. and Nagata, K. (2001) J. Vet. Med. Sci. 63, 115–124.
[15] Lele, Z., Hartson, S.D., Martin, C.C., Whitesell, L., Matts, R.L. and Krone, P.H. (1999) Dev. Biol. 210, 56–70.
[16] Yamada, T., Hashiguchi, A., Fukushima, S., Kakita, Y., Umezawa, A., Maruyama, T. and Hata, J. (2000) In Vitro Cell Dev. Biol. Anim. 36, 139–146.
[17] Nakai, A. and Ishikawa, T. (2001) EMBO J. 20, 2885–2895.
[18] Voss, A.K., Thomas, T. and Gruss, P. (2000) Development 127, 1–11.
[19] Hilscher, B., Hilscher, W., Buthoff-Ohnolz, B., Kramer, U., Birke, A., Pelzer, H. and Gauss, G. (1974) Cell Tissue Res. 154, 443–470.
[20] Gruppi, C.M., Zakeri, Z.F. and Wolgemuth, D.J. (1991) Mol. Reprod. Dev. 28, 209–217.
[21] Vanmuylder, N., Werry-Huet, A., Rooze, M. and Louryan, S. (2002) Anat. Embryol. 205, 301–306.
[22] Dugyala, R.R., Claggett, T.W., Kimmel, G.L. and Kimmel, C.A. (2002) Toxicol. Sci. 69, 183–190.
[23] Cambiazo, V., Gonzalez, M., Isamit, C. and Maccioni, R.B. (1999) FEBS Lett. 457, 343–347.
[24] Sreedhar, A.S. and Csermely, P. (2004) Pharmacol. Ther. (in press).
[25] Wu, Y.P., Kita, K. and Suzuki, N. (2002) Int. J. Cancer 100, 37–42.
[26] Zhang, S.L., Yu, J., Cheng, X.K., Ding, L., Heng, F.Y., Wu, N.H. and Shen, Y.F. (1999) FEBS Lett. 444, 130–135.
[27] Meng, X., Jerome, V., Devin, J., Baulieu, E.E. and Catelli, M.G. (1993) Biochem. Biophys. Res. Commun. 190, 630–636.

[28] Shen, Y., Liu, J., Wang, X., Cheng, X., Wang, Y. and Wu, N. (1997) FEBS Lett. 413, 92–98.
[29] Yost, H.J. and Lindquist, S. (1986) Cell 45, 185–193.
[30] Dale, E.C., Yang, X., Moore, S.K. and Shyamala, G. (1996) Gene 172, 279–284.
[31] Rebbe, N.F., Hickman, W.S., Ley, T.J., Stafford, D.W. and Hickman, S. (1989) J. Biol. Chem. 264, 15006–15011.
[32] Wu, J.M., Xiao, L., Cheng, X.K., Cui, L.X., Wu, N.H. and Shen, Y.F. (2003) J. Biol. Chem. 278, 51143–51149.
[33] Yufu, Y., Nishimura, J. and Nawata, H. (1992) Leukoc. Res. 16, 597–605.
[34] Jerome, V., Vourch, C., Baulieu, E.E. and Catelli, M.G. (1993) Exp. Cell Res. 205, 44–51.
[35] Jerome, V., Leger, J., Devin, J., Baulieu, E.E. and Catelli, M.G. (1991) Growth Factors 4, 317–327.
[36] Bertram, J., Palfner, K., Hiddemann, W. and Kneba, M. (1996) Anticancer Drugs 7, 838–845.
[37] Sreedhar, A.S., Mihály, K., Pató, B., Schnaider, T., Steták, A., Kis-Petik, K., Fidy, J., Simonics, T., Maráz, A. and Csermely, P. (2003) J. Biol. Chem. 278, 35231–35240.
[38] Liu, X., Ye, L., Wang, J. and Fan, D. (1999) Chin. Med. J. 112, 1133–1137.
[39] Ogata, M., Naito, Z., Tanaka, S., Moriyama, Y. and Asano, G. (2000) J. Nippon Med. Sch. 67, 177–185.
[40] Jameel, A., Skilton, R.A., Campbell, T.A., Chander, S.K., Coombes, R.C. and Luqmani, Y.A. (1992) Int. J. Cancer 50, 409–415.
[41] Gress, T.M., Muller-Pillasch, F., Weber, C., Lerch, M.M., Friess, H., Buchler, M., Beger, H.G. and Adler, G. (1994) Cancer Res. 54, 547–551.
[42] Twomey, B.M., Dhillon, V.B., McCallum, S., Isenberg, D.A. and Latchman, D.S. (1993) J. Autoimmun. 6, 495–506.
[43] Kamal, A., Thao, L., Sensintaffar, J., Zhang, L., Boehm, M.F., Fritz, L.C. and Burrows, F.J. (2003) Nature 425, 407–410.
[44] Neckers, L. (2003) Curr. Med. Chem. 10, 733–739.
[45] Soga, S., Kozawa, T., Narumi, H., Akinaga, S., Irie, K., Matsumoto, K., Sharma, S.V., Nakano, H., Mizukami, T. and Hara, M. (1998) J. Biol. Chem. 273, 822–828.
[46] Chiosis, G., Lucas, B., Shtil, A., Huezo, H. and Rosen, N. (2002) Bioorg. Med. Chem. 10, 3555–3564.
[47] Sreedhar, A.S., Soti, C. and Csermely, P. (2004) Biochim. Biophys. Acta (in press).
[48] Marcu, M.G., Chadli, A., Bouhouche, I., Catelli, M. and Neckers, L.M. (2000) J. Biol. Chem. 275, 37181–37186.
[49] Sõti, Cs., Rácz, A. and Csermely, P. (2002) J. Biol. Chem. 277, 7066–7075.
[50] Garnier, C., Lafitte, D., Tsvetkov, P.O., Barbier, P., Leclerc-Devin, J., Millot, J.M., Briand, C., Makarov, A.A., Catelli, M.G. and Peyrot, V. (2002) J. Biol. Chem. 277, 12208–12214.
[51] Sõti, Cs., Vermes, A., Haystead, T.A. and Csermely, P. (2003) Eur. J. Biochem. 270, 2421–2428.
[52] Passarino, G., Cavalleri, G.L., Stecconi, R., Franceschi, C., Altomare, K., Dato, S., Greco, V., Cavalli Sforza, L., Underhill, P.A. and de Benedictis, G. (2003) Hum. Mutat. 21, 554–555.