Cancer Letters 333 (2013) 133–146

Contents lists available at SciVerse ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Inhibition of the mitochondrial Hsp90 chaperone network: A novel, efficient treatment strategy for cancer?

Markus D. Siegelin *

Department of Pathology & Cell Biology, Columbia University College of Physicians & Surgeons, 630 W. 168th Street, VC14-239, New York, NY 10032, USA

ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 23 January 2013
Accepted 24 January 2013

Keywords:
Hsp90
Mitochondrial Hsp90 chaperone network
Shepherdin
Gamitrinibs
Apoptosis
Preclinical therapy

ABSTRACT

Research has shown that cancer cells exhibit multiple deregulated pathways, involving proliferation, migration and cell death. Heat-shock-proteins have evolved as "central regulators" and are implicated in the modulation of these pathways and in organelle-specific signaling. In this instance, heat-shock-proteins (Hsps) assist cancer cells in the maturation of proteins. Hsp90 is of particular interest because its enzymatic ATPase activity is elevated in malignant cells as compared to non-neoplastic counterparts. Consistent with its high-activity in cancer cells, Hsp90 stabilizes a considerable number of proteins being instrumental in carcinogenesis and the maintenance and growth of highly malignant cancers. Among its distribution Hsp90 is also localized within mitochondria of neoplastic cells of various origin, interacting with another chaperone, TRAP1 (Tumor necrosis factor type 1 receptor-associated protein or Heat-shock-protein 75) to antagonize the cell death promoting properties of the matrix protein, Cyclophilin-D. Several preclinical studies, including *in vivo* studies in both orthotopic and genetic animal models, have confirmed that targeting mitochondrial Hsp90 may be a novel efficient treatment method for highly recalcitrant tumors. This review summarizes the most recent findings of mitochondrial Hsp90 signaling and its potential implications for cancer therapy.

1. Introduction

Despite cardiovascular related deaths cancer is one of the most leading causes of death in the western world. According to the American Cancer Society (ACS) approximately 301.820 men and 275.370 women will succumb to cancer in the year 2012. The leading cancer deaths are caused by lung, prostate and colorectal cancer in adult men and by lung, breast and colorectal cancer in adult women (ACS). Adding up these numbers of both genders yields a significant total number of 577.190 cancer related deaths, equaling the size of an intermediate to large city in Europe. Considering the economic impact of cancer on the society the cost of the cancer is considered to be 190 billion US Dollars [1]. Since the 1970 the "Five-year relative Survival" has increased from 49% (1975–1977) to 67% (2001–2007) possibly caused by earlier detection of disease, significant advances in science and patient management. Although our understanding of the underlying biology has significantly risen in the past decades, there is still an urgent need for novel therapeutic therapies. In particular, certain cancers harbor a dramatic dismal prognosis with life expectancies often less than a year despite modern state-of-the art treatment regimes. Two prominent members out of this group are pancreatic and brain cancer. With respect to brain cancer, malignant gliomas are the most common primary brain tumors with glioblastoma multiforme WHO IV being the most common one [2,3]. Glioblastoma multiforme is characterized by highly pleomorphic astrocytic cells that tend to diffusely infiltrate the adjacent uninvolved healthy normal brain tissue, which in turn makes surgical resection impossible. In addition, the presence of the blood–brain-barrier [4] is a major obstacle for chemotherapeutics to reach the singly, dispersed highly migratory [5] and malignant glioma cells, which demonstrate significant heterogeneity [6]. With regards to its life-expectancy and tumor cell migration capacity, pancreatic cancer is reminiscent of glioblastoma to some extent. Arising most commonly in the pancreatic head and thereby causing bile duct obstruction, the invasive adenocarcinoma of the pancreas has a tendency to invade into the

Abbreviations: 17-AAG, 17-N-Allylamino-17-demethoxygeldanamycin; AMPK, AMP-activated kinase; CCCP, carbonyl cyanide 3-chlorophenylhydrazone; CsA, Cyclosporin A; CyD, Cyclophilin-D; G-G4, Gamitrinib(s), Geldanamycin (GA), mitochondrial matrix inhibitors – Gamitrinib – G4; G-TPP, Gamitrinib-TPP; HK-II, Hexokinase-II; Hsp, Heat-shock-protein; Hsp70/90, Heat-shock-protein 70/90; MGMT, O6-methylguanine-DNA-methyltransferase; mtUPR, mitochondrial unfolded protein response; MMR, mismatch repair proteins; MSI, microsatellite instability; MTP, mitochondrial transition pore; PKM2, pyruvatkinase isoform M2; TRAIL, TNF-related apoptosis-inducing ligand (TRAIL); TMZ, Temozolomide; TRAMP model, Transgenic Adenocarcinoma of the Mouse Prostate model; VDAC, voltage dependent anion channel.

* Tel.: +1 774 8239187.
E-mail address: ms4169@columbia.edu

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http://dx.doi.org/10.1016/j.canlet.2013.01.045

peri-pancreatic fat and the duodenum. Furthermore, pancreatic cancers often reveal marked desmoplasia surrounding the tumor cells, causing a significant inhibition of drug diffusion to the tumor cells, which ultimately make these tumors therapeutically refractive against a broad range of compounds [7]. In light of these numbers and limited therapeutic options new treatment regimens are highly necessary.

After deciphering the genetic code society had great hopes that this information will give rise to novel, sophisticated, tumor-specific targeted therapies in patients. These therapies were also phrased as "magic-bullets" [8,9] meaning that when a mutation or genetic alteration is found a compound targeting this genetic alteration would eradicate the tumor while healthy tissue (not carrying the genetic alteration) would be unaffected. Examples of this strategy include targeting the bcr-abl fusion protein (by Imatinib (Gleevec)) or the BRAF V600E mutation (by Vemurafenib). A recently completed phase III clinical trial in melanoma, involving 675 patients, with Vemurafenib led to promising results [10,11]. In this trial, melanoma patients were randomly assigned to a treatment with the established chemotherapeutic drug, dacarbazine or the BRAF inhibitor, Vemurafenib. Patients with melanomas harboring a V600E BRAF mutation that were subjected to Vemurafenib treatment revealed a significant improved overall as well as progression-free survival when compared to the dacarbazine treatment group [10,11]. In the light of the dismal prognosis of melanoma these results represent truly a significant advancement to the field of cancer research and particularly to personalized medicine. Recently developed "mutation-specific" antibodies can easily identify certain molecular changes, such as BRAF mutations. In fact, a novel antibody that detects the BRAF V600E mutation has been recently developed [12] and it has been reported that this antibody reveals a high sensitivity and specificity to recognize the BRAF V600E mutation in a number of malignancies, including papillary thyroid carcinoma [12], melanoma [12], gliomas, ovarian carcinomas [13] and Langerhans and non-Langerhans cell histiocytosis [14,15]. The true advantage with such novel tools is that tumor tissues can be analyzed in a less labor-intensive and more cost-effective manner compared to regular sequencing-approaches. Despite the excitement with such novel treatment avenues tumors cells find ways to develop additional means to entertain their growth. Among several reasons, tumors regardless of their origin usually harbor a great number of genetic alterations in multiple pathways, suggesting that these "magic-bullets" targeting one specific pathway or genetic alteration will fall short of expectations as tumor cells may switch to another deregulated pathway [16]. Considering the low-success rate and high-costs of these "magic bullet" treatments and the high threshold to transfer a drug from the bench into patient management, clinical trials have in most cases not yielded dramatic improvements in patients' survival or life quality [16]. Therefore new strategies to combat high-resistant cancers need to be elucidated.

As described earlier it becomes more and more obvious that cancer cells exhibit multiple deregulated pathways, involving proliferation, migration and cell death, e.g. apoptosis, autophagy and necrosis. Heat-shock-proteins (Hsps) are bona-fide prototypes of "central regulators" that control the above-mentioned cellular functions and properties. Generally, they assist cells in the maturation of proteins, for instance in folding and re-folding of proteins [17]. Since cancer cells are commonly exposed to stressors [18], such as hypoxia, nutrient deprivation and various aggressive treatments, e.g. radiation and chemotherapy, this may result in a "stress response" in cancer cells, culminating in the accumulation of unfolded proteins in several organelles (cytosol, endoplasmatic reticulum and mitochondria). Ultimately, this stress response may result in necrotic, apoptotic or autophagic cell death [19]. To compensate cancer cells rely on Hsps to account for these environmental changes [19]. One common pathway, which is activated in cells exposed to stress is the HSF1 pathway, regulating and driving the transcription and expression of heat-shock-protein 70 (Hsp70) [20]. In addition, HSF1 modulates apoptosis, proliferation, protein synthesis and glucose metabolism, which are all important factors in the regulation of cancer growth [20]. In transgenic animal models mutations of the RAS oncogene or TP53 are dependent on HSF1 to drive carcinogenesis [20].

With regards to its anti-apoptotic functions Hsp70 interferes with apoptotic signaling at multiple levels of the intrinsic apoptotic pathway, which requires its ATPase activity and the presence of a C-terminal EEVD sequence [21]. Requiring a functional ATPase acitivty, Hsp70 is known to inhibit cytochrome-c release from mitochondria and in concert with Hsp90 also binds to Apaf-1 that is required for the apoptosome formation in the cytosol to activate Pro-caspase-9 [22]. Furthermore, Hsp70 interacts with AIF (apoptosis inducing factor) to inhibit its release from the mitochondria into the cytosol and nuclear import [22]. It has also been recently shown that the anti-apoptotic transcription factor ATF5 [23–25], which controls the expression of Bcl-2, Mcl-1 and mTOR [25], is stabilized by Hsp70 [26] The JNK pathway is of particular interest when it comes to the modulation of apoptotic sensitivity in cancer cells and Hsp70 possesses the ability to modulate JNK activity in cancer cells independent of its ATPase activity [21].

Among the Hsps Heat-shock-protein 90 (Hsp90) is of particular interest that is over-expressed in cancer cells and its N-terminal ATPase activity is approximately 100 fold elevated in malignant cells of various origin when compared to non-neoplastic counterparts [27]. This enables Hsp90 to tightly bind interacting partners in a "high-affinity conformation" [27]. Consistent with its high-activity in cancer cells of various origin, Hsp90 stabilizes a considerable number of proteins being involved in carcinogenesis and the maintenance of highly-malignant anaplastic cells [17], e.g. survivin, Akt (and its phosphorylated forms), p53 (mutated and non-mutated), EGFR (including mutated forms), HER2 [17], ALK [28], BRAF (also the mutated V600E BRAF) and Bcr-abl (summarized nicely in [29]). A lot of these mutated kinases dependent on the active interaction and chaperoning by Hsp90 since otherwise the fate of these molecules would be fast and efficient degradation by the proteasome.

Inhibition of heat-shock-protein 90 has been accomplished by a molecule called geldanamycin. Due to its toxic effects geldanamycin has been chemically modified to give rise to 17-AAG (tanespimycin). 17-AAG is less toxic and is being exploited in clinical trials (www.clinicaltrials.gov) in multiple malignancies since 1999 [17]. Despite its non-toxic pharmacological profile and that it is well-tolerated in patients, its anti-cancer activity in patients is unfortunately mild to modest [17]. This mild efficacy might be explained well by pharmaco-dynamic issues, but other factors are also likely to contribute to treatment resistance. One main consequence of the inhibition of heat-shock-protein 90 is the concomitant up-regulation of this interacting partner, heat-shock-protein 70, Hsp70 [30]. In fact, Hsp70 up-regulation is a surrogate marker for Hsp90 inhibition [31]. An unfortunate accompanying effect about Hsp70 up-regulation is that Hsp70 itself drives proliferation and inhibits apoptosis in cancer cells through multiple mechanisms. Therefore, it would be desirable to acquire Hsp90 inhibitors that are devoid of the accompanying Hsp70 up-regulation, e.g. gamitrinibs [17,31] see below. Another viable alternative would be to combine Hsp90- with Hsp70 inhibitors [32]. Powers et al. [32] supported this hypothesis in a report, which demonstrated that susceptibility of cancer cells to 17-AAG is determined by specific HSP70 isoforms.