DOWNLOAD PDF TARGETING HSP90: THE CANCER SUPER-CHAPERONE PAUL WORKMAN AND SWEE SHARP.

Chapter 1 : Publications Authored by Swee Sharp | PubFacts

Abstract. A series of benzo-macrolactones has been prepared by chemical synthesis, and evaluated as inhibitors of heat shock protein 90 (Hsp90), an emerging attractive target for novel cancer therapeutic agents.

HSP90 as a cancer drug target As our understanding of the genetic and molecular biology of cancer has increased, there has been a shift over the last decade in the approaches used in the discovery of novel cancer therapeutics Workman In contrast to the earlier development of cytotoxic agents, focus has moved to the development of treatments that target the pathways responsible for malignancy. Validation of this approach has been provided by the clinical activity and approval of small-molecule kinase inhibitors such as imatinib Gleevec , gefitinib Iressa and erlotinib Tarceva as well as the therapeutic antibodies trastuzumab Herceptin , cetuximab Erbutix and bevacizumab Avastin. However, despite the success that these agents have enjoyed, it is likely that modulation of a single molecular target will be insufficient for optimal therapy Workman Even where malignancies are driven by single genes or pathways, the development of resistance is a major concern. For example, resistance to imatinib has been shown to arise by acquisition of mutations within the kinase domain of BCRâ€”ABL Gorre et al. Furthermore, the majority of cancers involve multiple molecular abnormalities that are likely to be involved in malignant progression. These observations have reinforced the suggestion that inhibition of multiple targets will be required to cure most human cancers Workman It is this concern which provides the foundation for the increasing amount of interest in targeting the heat shock protein 90 HSP90 molecular chaperone Workman Of particular importance is that HSP90 is essential for the stability and the function of many oncogenic client proteins, which contribute to the hallmark traits of cancer Fig. Inhibition of HSP90 function has been shown to cause degradation of client proteins via the ubiquitin-proteasome pathway Connell et al. The ability to deliver a combinatorial effect through a single drug target may have promise in treating cancers driven by multiple molecular abnormalities and could also reduce the opportunity for resistance developing Workman , In this paper, we will focus on our interest in developing inhibitors of the HSP90 molecular chaperone family and the progress we have made in understanding the effects of this modulation in both the preclinical and clinical settings. Examples will be taken mainly from the work of our own laboratory. Presently, five isoforms of HSP90 have been identified, which differ in their cellular localisation. The chaperone activity of HSP90 is dependent on its transient N-terminal dimerisation, which stimulates the intrinsic and essential ATPase activity Prodromou et al. This process is controlled by an orchestrated set of interactions with a range of accessory proteins referred to as co-chaperones Pratt et al. The present model for chaperone activity suggests that when HSP90 exchanges ADP for ATP, it undergoes a conformational change, which includes the transient dimerisation of the N-terminal domains Prodromou et al. Inhibition of ATP binding to HSP90 prevents the formation of the mature complex and results in the proteasome-dependent degradation of associated client proteins. Both of these molecular chaperones may be present in the immature complex, which has been stabilised by the presence of an inhibitor Fig. At first sight, HSP90 would not appear to be an obvious drug candidate for the design of novel cancer therapeutics. This is because it is not, to our knowledge, subject to mutation or amplification in cancer. This may be a consequence of the hostile conditions created in tumour cells by the effects of deregulated oncogenes and tumour suppressor genes many of which are HSP90 client proteins , along with the stressful microenvironmental features of solid tumours, which include nutrient deprivation, hypoxia and acidosis Whitesell et al. This will increase the opportunity for therapeutic selectivity when HSP90 inhibitors are used clinically. In addition to the stressed nature of cancer cells, HSP90 inhibitors could exert therapeutic selectivity by exploiting multiple oncogene addiction and via the preferential dependence of certain oncoproteins on chaperoning by HSP90 see later. It is known that the natural product geldanamycin exerts its antitumour effect by binding to the N-terminal ATPase domain of HSP90 to inhibit its chaperone function Roe et al. The progress of geldanamycin into the clinic was stopped due to instability and the unacceptable hepatotoxicity seen at

therapeutic doses during preclinical in vivo studies Supko et al. Further analogues were developed for clinical use, which included AAG Schnur et al. Preclinical studies with AAG We have investigated the detailed molecular consequences of exposing cancer cells to AAG in vitro in an attempt to identify genes and proteins that influence the sensitivity to HSP90 inhibitors. In addition, these studies have enabled us to identify and validate biomarkers of HSP90 inhibition, which could be of clinical use Banerji et al. It has been well documented that inhibition of HSP90 function induces the expression of HSP72 and degradation of client proteins reviewed in Workman , Clarke et al. We have demonstrated the principle of combinatorial inhibition of multiple signal transduction pathways via targeting HSP90 using a panel of human colon cancer cell lines Hostein et al. We showed that this was accompanied by cytostasis, cell-cycle arrest and cell-line-dependent apoptosis Hostein et al. The extent of the apoptotic effect was hypothesised to be influenced by the expression of the proapoptotic BCL2 family member, BAX. We and other researchers have subsequently used an isogenic pair of HCT cells in which the BAX gene has been removed by homologous recombination Zhang et al. Using this approach, we showed that ERBB2 overexpression resulted in a fivefold increase in sensitivity to geldanamycin Smith et al. In addition, we have recently demonstrated that in human ovarian cancer cells, which can become resistant to conventional cytotoxics e. These observations highlight the potential use of AAG both as a single agent and as a potential sensitiser to current chemotherapeutic agents. Consistent with the proposed combinatorial action of HSP90 inhibitors on the hallmarks of cancer, we and other researchers have recently demonstrated the profound effects of AAG and geldanamycin on key aspects of tumour angiogenesis and potentially also of lymphangiogenesis de Candia et al. These molecular changes were accompanied by reduced endothelial cell migration, tubular differentiation, invasion through Matrigel and secretion of urokinase-type plasminogen activator in human endothelial cells in response to treatment with geldanamycin and its derivatives Sanderson et al. Collectively, our results suggest that effects on angiogenesis may play a considerable role in the response to HSP90 inhibitors in vivo. To further our understanding of the molecular changes associated with HSP90 inhibition, we conducted the first cDNA expression profiling analysis of a human colon cancer cell line panel following AAG treatment Clarke et al. The induction of HSP70 family members observed in this study and elsewhere Hostein et al. The direction of this modulation correlated with the cellular sensitivity to AAG. HSP90 is itself antiapoptotic and we have, therefore, hypothesised that the alteration of target expression by the drug may be a factor influencing the cellular sensitivity to AAG. The expected changes described above were shown to be reproducible in this cell line, but at an even greater level of detail than was demonstrated previously. However, a novel and important observation was the altered expression of a group of proteins involved in chromatin regulation, acetylation and methylation in response to AAG A Maloney et al. These observations, along with the decrease in total cell acetylation observed during these studies in response to AAG suggest that there is an interaction between protein acetylation and HSP90 function. This is reinforced by other studies, which have suggested that HSP90 function may be inhibited by histone deacetylase inhibitors via a direct increase in acetylation of the chaperone Yu et al. Clinical evaluation of AAG Based on the promising preclinical activity of AAG, we carried out one of the initial phase I clinical trials, which provided the first convincing proof-of-concept for HSP90 inhibition in human patients Banerji et al. Molecular biomarkers that we and other researchers selected for use during the clinical trial included the induction of HSP72 and depletion of HSP90 client proteins C-RAF and CDK4 in the tumour tissue and peripheral blood lymphocytes of treated patients Banerji et al. These biomarkers were validated in studies of pharmacodynamicâ€”pharmacokinetic relationships carried out in a human ovarian xenograft model Banerji et al. We were able to confirm, using pharmacokinetic analysis, that AAG was present at therapeutic plasma concentrations after i. Encouragingly, we observed prolonged stable disease in two patients with advanced, metastatic malignant melanoma, which led to a phase II clinical trial being initiated at our institution and the Royal Marsden Hospital in collaboration with the Royal Free Hospital, London. In addition to melanoma, evidence of clinical activity has also been reported by others in breast and prostate cancer Pacey et al. To understand the sensitivity of melanoma cells to AAG, mechanistic

studies were carried out, in particular to investigate the hypothesis that melanoma responsiveness may be related to the high incidence of B-RAF mutation in this disease Davies et al. However, it should be noted that we saw no relationship between B-RAF status and sensitivity in melanoma cell lines. This may be because C-RAF is also a sensitive client protein. Similar results have been obtained in an independent study Grbovic et al. Overall, our phase I clinical trial demonstrated that it was possible to deliver a dose of AAG, using a once weekly schedule, which achieved potentially therapeutic plasma concentrations, HSP90 target inhibition in tumour tissue and possible antitumour activity Banerji et al. However, the cumbersome formulation of AAG prevented the true evaluation of its maximum tolerated dose. Nevertheless, phase II studies are underway and combination studies are in progress; some of these involve alternative formulations Pacey et al. However, in addition to its poor solubility and cumbersome formulation, this compound does have additional limitations, which have provided a strong case for the development of improved second generation inhibitors. These include relatively weak target potency, reduced activity in the presence of P-glycoprotein Kelland et al. We proposed that this was due to metabolism to a more active HSP90 inhibitor. Recent studies have confirmed that this increase in potency is indeed due to the metabolism of AAG to the more active hydroquinone form Guo et al. By studying different analogues of AAG, we were able to show that the NQO1 potentiation effect was restricted to alkyl-substituted amino analogues of geldanamycin Kelland et al. Therefore, it may be possible to circumvent the solubility and formulation issues seen with AAG. Clinical trials by ourselves and other researchers have been initiated using this novel derivative Hollingshead et al. The wide array of HSP90 inhibitors currently in the preclinical pipeline has been reviewed extensively elsewhere Chiosis et al. The first generation of synthetic small-molecule HSP90 inhibitors were purine analogues Chiosis et al. From this, more potent analogues were designed, which demonstrated at least 50 times more activity than PU3 Wright et al. Our progress in developing novel HSP90 inhibitors was furthered by a high-throughput screen of our then 53 compound library using a malachite green assay to measure the ATPase activity of the full-length recombinant yeast HSP90 Aherne et al. Using this screen, we identified the diaryl pyrazole resorcinol series of HSP90 inhibitors exemplified by the initial micromolar hit CCT Cheung et al. As part of a productive collaboration with Vernalis, structure-guided introduction of the 5-amide substitution increased hydrogen bond interaction with Gly of human HSP90 and led to the generation of the more potent analogues. This is exemplified by VER CCT , which compares favourably with AAG, has nanomolar activity and has the required potency and potential to become a clinical candidate Dymock et al. We have demonstrated that CCT inhibits a range of different cancer cell lines in vitro at micromolar concentrations, which caused degradation of client proteins and induction of HSP This molecular biomarker signature of HSP90 inhibition was accompanied by cell cytostasis, G1 cell-cycle arrest and apoptosis SY Sharp et al. In agreement with our earlier studies using geldanamycin and its derivatives, CCT was observed to reduce tumour cell invasion and exhibit antiangiogenic activity Sharp et al. It was concluded from these studies that the diaryl pyrazole resorcinol series of novel HSP90 inhibitors have similar cellular properties to AAG, but have several possible advantages e. These studies demonstrate that high-throughput screening combined with X-ray crystallography and structure-based design can provide a powerful approach for the discovery of HSP90 ATPase inhibitors. Novel approaches to inhibit the HSP90 molecular chaperone The results from our laboratory and others discussed so far have focused on the effect of inhibiting the ATPase activity of HSP90 activity directly. However, as described earlier, HSP90 functions in a multi-protein complex comprised of client proteins and co-chaperones. These data have led us to propose that AHA1 may be an interesting drug target with potential inhibitors being used either alone or alongside existing HSP90 modulators to improve their therapeutic efficacy. As discussed earlier, induction of HSP70 isoforms has been shown by our laboratory and others to occur in response to AAG both in vitro and in vivo Hostein et al. This was accompanied by inhibition of cell growth and induction of cell death, the extent of which was far greater than is observed with AAG. Concluding remarks HSP90 is an exciting new therapeutic target, inhibition of which delivers a combinatorial attack on multiple oncogenic targets and pathways and on all of the hallmark traits of malignancy. The development of HSP90 inhibitors

has moved forward rapidly alongside our growing understanding of the role of the chaperone in normal and malignant cells. Following on from the natural product-based agents, exemplified by AAG and related analogues that have entered clinical trials, a variety of HSPinhibitory chemo-types are now under development. It is also possible that new classes of inhibitor could be developed which act upon the co-chaperones of HSP Clinical activity has been seen with AAG in melanoma, breast and prostate cancer. Although a strength of HSP90 inhibitors is their combinatorial action in depleting multiple client proteins, this can, at the same time, obscure the precise mechanism of action that may predominate in a particular cancer. Action on these and other client proteins, such as mutant B-RAF, provides the potential for activity in a wide range of cancers. Download Figure Figure 1 Schematic illustrating how inhibition of HSP90 may interfere with all of the six hallmark traits of cancer. Examples of client proteins involved in the various phenotypic aspects of malignancy are shown.

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The molecular chaperone heat shock protein 90 (HSP90) has emerged as an exciting molecular target for cancer therapy. It operates as part of a multichaperone complex and is essential for the.

Chapter 3 : Inhibitors of Molecular Chaperones as Therapeutic Agents (RSC Publishing)

Through its chaperoning activity, HSP90 is a master regulator of many cancer-associated proteins. Inhibitors of HSP90 exhibit the unique ability to disrupt oncogenic signalling networks at multiple levels.

Chapter 4 : - NLM Catalog Result

Targeted therapy is one of the most burgeoning areas of development in cancer research. Increasing knowledge in tumor formation and growth at the molecular.