Bioorganic & Medicinal Chemistry Letters 24 (2014) 661–666

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

A heat shock protein 90 inhibitor that modulates the immunophilins and regulates hormone receptors without inducing the heat shock response

Jeanette R. McConnell [a,†], Leslie A. Alexander [b], Shelli R. McAlpine [a,*,†]

[a] Department of Chemistry, University of New South Wales, Kensington, NSW 2052, Australia

[b] Department of Chemistry and Biochemistry, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-1030, United States

ARTICLE INFO

Article history:
Received 22 November 2013
Accepted 22 November 2013
Available online 1 December 2013

Keywords:
Heat shock protein 90 (hsp90)
Tetratricopeptide-repeat (TPR)
FKBP51
FKBP52
Heat shock response
Heat shock factor 1 (HSF1)

ABSTRACT

When a cell encounters external stressors, such as lack of nutrients, elevated temperatures, changes in pH or other stressful environments, a key set of evolutionarily conserved proteins, the heat shock proteins (hsps), become overexpressed. Hsps are classified into six major families with the hsp90 family being the best understood; an increase in cell stress leads to increased levels of hsp90, which leads to cellular protection. A hallmark of hsp90 inhibitors is that they induce a cell rescue mechanism, the heat shock response. We define the unique molecular profile of a compound (SM145) that regulates hormone receptor protein levels through hsp90 inhibition without inducing the heat shock response. Modulation of the binding event between heat shock protein 90 and the immunophilins/homologs using SM145, leads to a decrease in hormone receptor protein levels. Unlike N-terminal hsp90 inhibitors, this hsp90 inhibitor does not induce a heat shock response. This work is proof of principle that controlling hormone receptor expression can occur by inhibiting hsp90 without inducing pro-survival protein heat shock protein 70 (hsp70) or other proteins associated with the heat shock response. Innovatively, we show that blocking the heat shock response, in addition to hsp90, is key to regulating hsp90-associated pathways.

© 2013 Elsevier Ltd. All rights reserved.

When a cell encounters external stressors, such as lack of nutrients, elevated temperatures, changes in pH or other stressful environments, a key set of evolutionarily conserved proteins, the heat shock proteins (hsps), become overexpressed.[1–7] The hsps are molecular chaperones broken down into six distinct families based on their molecular size (hsp100, hsp90, hsp70, hsp60, hsp40 and the small hsps).[8] While all of these hsps are important in normal cells and become overexpressed in stressed cells, hsp90 is the most prominent. In an unstressed cell, hsp90 makes up 1–2% of the total protein load, and upon external stressors this is increased to 3–5%.[9] One major stressor known to induce this upregulation of hsp90 is malignancy.[2,10–15] The large amount of mutated and mis-folded proteins in cancer cells cause them to become dependent upon the molecular chaperone activity of hsp90; because hsp90 protects the function of more than 200 client proteins, many of which are associated with oncogenesis (Fig. 1).[1,2,4,16–18] Thus, cancer cells are significantly more dependent on hsp90 than normal cells.[19]

Hsp90 is ATP dependent, functional only when dimerized and broken down into 3 domains, the amino (N), middle (M), and carboxy (C) domains (Fig. 1). The C-terminal domain is known to interact with a specific subset of proteins that contain a tetratricopeptide-repeat (TPR) domain.[20] The TPR domain is a protein scaffold consisting of a semi-conserved sequence of 34 amino acids that occur in repeats throughout the protein.[21] Within the group of sixteen TPR proteins that interact with hsp90, four are immunophilins: FK506 binding protein 52 (FKBP52), FKBP51, cyclophilin 40 (Cyp40), and FKBP38. There are also several are homologs including: C-terminus of Hsc70 interacting protein (CHIP), Unc45, and mitochondrial import receptor of 70 kDa (Tom70).[22] In addition, a key co-chaperone that regulates hsp90's function is the TPR-containing heat shock organizing protein (HOP). These TPR-containing proteins are all regulated via their interaction with hsp90's MEEVD region (M = methionine, E = glutamic acid, V = valine, D = aspartic acid), located at the C-terminus (Fig. 1).

Three of the four immunophilins (FKBP51, FKBP52 and Cyp40) are well established to regulate cell growth through controlling hormone receptor (HR) interactions with hsp90.[23] In addition, the homologs CHIP, Unc45 and Tom70 facilitate hormone

Abbreviations: DMSO, dimethyl sulfoxide; IC_{50}, inhibitory concentration (50%).

* Corresponding author. Present address: School of Chemistry, University of New South Wales, Sydney, NSW 2052, Australia. Tel.: +61 2 9385 5505.

E-mail address: s.mcalpine@UNSW.edu.au (S.R. McAlpine).

† Tel.: +61 4 1672 8896; fax: +61 2 9385 6111.

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.11.059

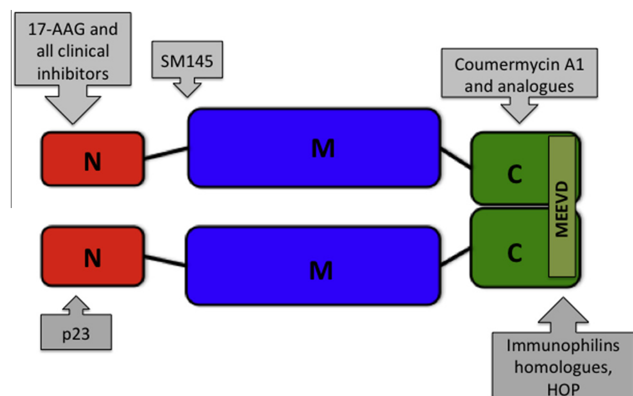

Figure 1. Hsp90 cartoon depiction. The hsp90 dimer indicating where 17-AAG, coumermycin A1, SM145, and various co-chaperones important in hormone receptor development bind (M = methionine, E = glutamic acid, V = valine, D = aspartic acid).

receptor-regulated cell growth via hsp90.[23] These co-chaperones regulate the maturation of hormone receptors by forming a multi-chaperone complex with hsp90 and the co-chaperone p23. This complex induces a signaling cascade leading to cell growth.[23] Since the interaction of hsp90 with the immunophilins regulates HR development, blocking the interaction between the immunophilins' TPR domain and hsp90's MEEVD region will likely affect HR protein levels. Since hsp90 and its co-chaperones regulate HR maturation, targeting this pathway may avoid existing cell rescue mechanisms.

There are two general classes of hsp90 inhibitors that have been extensively investigated: those that bind to the N-terminus and those that bind to the C-terminus of hsp90. There are 4 inhibitors in clinical development and they all bind to the ATP-binding pocket at the N-terminal domain. Of these four drugs, three are structurally related and contain a resorcinolic acid motif (similar to NVP-AUY992) and the fourth is an analogue of 17-AAG, which has been previously clinically tested and removed due to toxicity (Fig. 2). These four drugs all impact the same signaling pathways and have no impact on the binding between the immunophilins and hsp90. Thus, they are ineffective tools for delineating the relationship between the immunophilins, hsp90, and HR production. Furthermore, the hsp90 inhibitors currently in clinical trials are combating drug resistance, which is caused by the activation of the heat shock response (HSR).[24,25] The HSR is a cell survival mechanism that induces the over-expression of hsp70 and other heat shock proteins that rescue the oncogenic pathways usually controlled by hsp90.[6]

The second class of molecules that bind to hsp90 are compounds that target its C-terminus. The most effective are coumermycin A1 (CA1) and its analogs (Fig. 2).[20,26,27] There is currently no data on how the C-terminal hsp90 inhibitor CA1 controls the immunophilins. However, work done by Ratajczak and co-workers showed that a millimolar concentration of a structurally similar C-terminal inhibitor, novabiocin, disrupts immunophilins from binding to hsp90.[28] Despite extensive research on hsp90 as a therapeutic target, there is a large and important knowledge gap within the hsp90 field: how do we inhibit hsp90 pathways without inducing a heat shock response?

Herein we describe the first small molecule, SM145, which binds at a novel site (between the N and middle domain of hsp90) and modulates binding between hsp90 and multiple immunophilins/homologs via blocking the interaction between MEEVD and TPR binding sites. We show that disrupting the association between hsp90 and the immunophilins leads to a decrease in hormone receptor protein levels without inducing the heat shock response (HSR). This work is proof of principle and the first example showing that directly inhibiting hsp90 can modulate the hormone receptor levels without inducing the pro-survival protein heat shock protein 70 (hsp70) or causing the HSR.

We have previously reported that SM145 binds to the NM-domain of hsp90, induces apoptosis and inhibits it's binding to specific client proteins and co-chaperones.[29–31] To prove that SM145 inhibits the molecular chaperone function of hsp90, we performed two types of luciferase refolding assays. The first was a pure protein assay, where hsp90 and necessary co-chaperones were incubated with heat-denatured luciferase. The second assay was a rabbit reticulocyte lysate (RRL, Promega) based luciferase refolding assay, where denatured luciferase was incubated in the complete system of the lysate. In the pure protein assay inhibition of luciferase activity can be tied directly to inhibiting hsp90. The second, lysate-based assay, shows that hsp90 inhibition also occurs in the more complex system of proteins that are normally involved in protein refolding. 17-AAG was used as a control.[32,33] Addition of SM145 to the pure protein system or to the RRL and subsequent measurement of luciferase activity showed that SM145 decreased

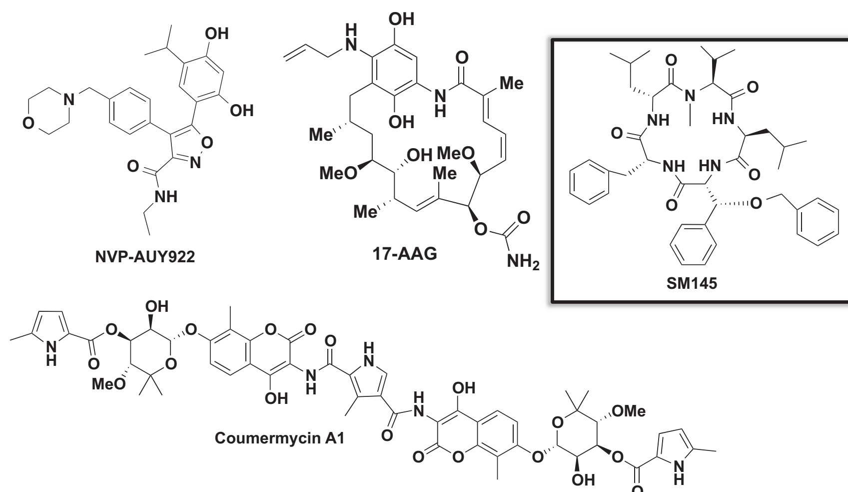

Figure 2. Structure of hsp90 inhibitors. SM145, 17-AAG (amino-terminal hsp90 inhibitor), NVP-AUY922 (resocinoic acid containing amino-terminal hsp90 inhibitor) and coumermycin A1 (carboxy-terminal hsp90 inhibitor).

Download English Version:

https://daneshyari.com/en/article/10592976

Download Persian Version:

https://daneshyari.com/article/10592976

Daneshyari.com