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editorial

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Finding Hsp90 inhibitors by drug repurposing: the power of chemical genetics

Heat shock protein 90 (Hsp90) is a promising anticancer target, because it regulates different phases of tumor progression, such as immortalization, impaired apoptosis, angiogenesis and invasion [1]. Since Hsp90 allows tumor cells to tolerate lethal genetic alterations that contribute to drug resistance, it is also of therapeutic importance as a means of controlling the development of resistance to anticancer drugs [2,3]. Thus, much effort has been devoted to finding Hsp90 inhibitors as anticancer drugs.

Three major strategies have been employed to screen Hsp90 inhibitors, including targeting the N-terminal ATP-binding pocket, the C-terminal nucleotide-binding domain, or by disrupting co-chaperone interactions [4]. To date, hundreds of Hsp90 inhibitors have been identified. None of them has been clinically approved. Those inhibitors that bind to the N-terminal ATP-binding site are the most effective agents, but they induce the heat shock response and exhibit anti-apoptotic and pro-survival effects [5], which limits their utility as anticancer agents. On the other hand, in cancer cells, Hsp90 primarily exists as a heteroprotein complex, which exhibits higher affinity for both ATP and ligands than the homodimeric form present in normal cells [6]. Since the cellular concentration of ATP is rather high, it is reasonable to infer that the inhibitor has to reach a relatively high concentration to compete with ATP for binding to Hsp90. This may be also responsible for the difficulty in finding efficient Hsp90 inhibitors as anticancer drugs. Therefore, it seems that it is time to shift the strategy in Hsp90-targeted drug discovery.

To meet the current 'more-investment-less-drug' challenge prevalent in today's pharmaceutical industry, drug repurposing or drug repositioning has attracted more and more attention and has been regarded as one of the most important strategies in translational medicine. Although various methods have been used to find new uses for existing drugs [7–9], the approaches derived from Connectivity Map (CMAP) analysis are of particular interest. CMAP is a chemical genetic database which collects microarray gene-expression profiles from cultured human cells treated with bioactive small molecules [10]. CMAP can be applied to systematically explore the functional connections among diseases, genetic perturbations and drug actions [10], which will provide very useful clues for drug repurposing. For instance, through integrating the gene expression profiles of 100 diseases and of 164 drugs recorded in CMAP database, Butte and co-workers repositioned cimetidine from antiulcer to anticancer (for lung adenocarcinoma) and has validated its effect by experiments [11]. Through comparing the similarities between chemical-associated gene expression profiles, Iorio *et al.* partitioned 1233 agents

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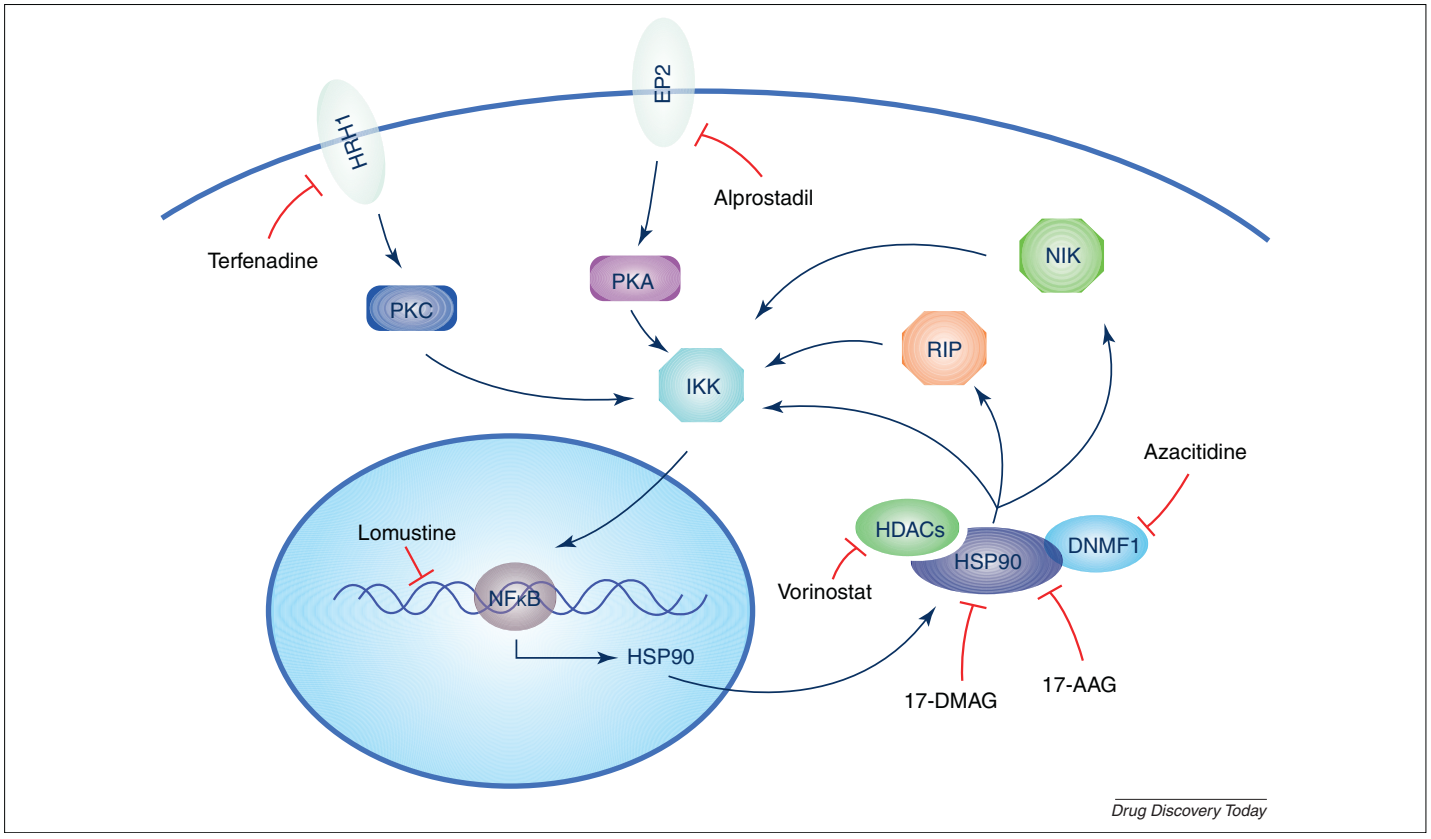

FIGURE 1

Approved drugs with potential Hsp90-inhibitory activities identified from CMAP database. The signature gene expression profiles of Hsp90 inhibitors 17-AAG or 17-DMAG were submitted into CMAP (build 02) for enrichment of potential Hsp90 inhibitors. Five approved drugs were revealed to have potential Hsp90-inhibitory activities, in which vorinostat, lomustine and azacitidine are anticancer drugs, alprostadil is an anti-vasodilator drug, and terfenadine is an anti-histamine drug.

into 106 communities [12]. The members of each community are likely to have similar pharmacological effects. It was found that fulvestrant, an approved estrogen receptor (ESR1) antagonist, was in the same community with Hsp90 inhibitors geldanamycin and tanespimycin, which suggests that fulvestrant may have Hsp90-inhibitory activity [12]. The preliminary success of CMAP-based drug repurposing stimulated our interest to explore whether we can find more approved drugs as Hsp90 inhibitors from CMAP.

The latest version of CMAP (build 02) (http://www.broad.mit.edu/cmap/) contains 6100 microarray expression profiles from cultured human cells treated with 1309 bioactive small molecules [10]. Kurashina *et al.* has identified 11 up-regulated and 17 down-regulated genes in three ATL cell lines (TaY-E10, MT-2, and MT-4) treated by Hsp90 inhibitors (i.e. 17-AAG or 17-DMAG), which defined gene expression profiles of Hsp90 inhibitors (Table S1) [13]. Through submitting these gene expression signatures into CMAP (build 02), 13 small molecules were enriched with high connectivity scores (permutation P-value < 0.005) (Table S2), which include five approved drugs, i.e. lomustine, vorinostat, azacitidine, alprostadil and terfenadine.

The targets of these approved drugs and their participated pathways associated with Hsp90 are illustrated in Fig. 1. It is interesting to note that although these drugs do not target Hsp90 directly, they might show similar activity as Hsp90 inhibitors through an indirect manner. For instance, it is well known that inhibition and/or knock-down of histone deacetylase 6 (HDAC6) induce Hsp90 acetylation, which decreases the binding ability of Hsp90 to ATP and thus prevents the formation of stable Hsp90-client protein complex [14]. As a result, HDAC inhibitors exhibit Hsp90-inhibitory activity [15]. DNA (cytosine-5)-methyltransferase 1 (DNMT1) is an Hsp90 client protein. Disruption of Hsp90 function leads DNMT1 to be unstable [16], which means that inhibition of DNMT1 brings similar effects as inhibition of Hsp90. Signal pathways regulated by histamine H1 receptor (HRH1) and prostaglandin E2 receptor subtype 2 (EP2) converged with those regulated by Hsp90 at the point of I-κ-B-kinase (IKK) [17–20]. This suggests that HRH1 and EP2 inhibitors display Hsp90-inhibitor-like activities.

In these agents, HDAC inhibitor vorinostat is of special interest, because it modulates the activity of Hsp90 in an indirect way. The indirect inhibition of Hsp90 has some advantages over the direct manners. First, indirect Hsp90 inhibitors do not compete with ATP. Second, according the CMAP database, we found that vorinostat does not up-regulate the expression of Hsp90 gene. In comparison, the direct inhibitors of Hsp90, i.e. geldanamycin and tanespimycin, induce a substantial up-regulation of Hsp90 gene expression. Therefore, it seems that the indirect Hsp90 inhibitors will be more efficient than the direct counterparts, which is in accordance with the new concept of network drug discovery that appeals to target malfunctioning proteins via other proteins [21].

In summary, thanks to the effort of chemical geneticists, rich information about chemical-associated gene expression profiles of human cell lines has been accumulated, which is of great value in drug repurposing. The present analysis indicates that the chemical genetic methodology is powerful in finding Hsp90-inhibitor-like agents from approved drugs. Notably, three of five identified agents, i.e. vorinostat, azacitidine and lomustine, are anticancer drugs. It is, thus, clearly of interest to evaluate whether the combination of these agents with other anticancer drugs has synergistic effects, in particular whether HDAC inhibitors have the activity of preventing anticancer drug resistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.drudis.2012.03.009.

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