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Allosteric Inhibition of the Protein-Protein Interaction between the Leukemia-Associated Proteins Runx1 and CBFβ

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SUMMARY

The two subunits of core binding factor (Runx1 and CBFβ) play critical roles in hematopoiesis and are frequent targets of chromosomal translocations found in leukemia. The binding of the CBFβ-smooth muscle myosin heavy chain (SMMHC) fusion protein to Runx1 is essential for leukemogenesis, making this a viable target for treatment. We have developed inhibitors with low micromolar affinity which effectively block binding of Runx1 to CBFβ. NMR-based docking shows that these compounds bind to CBFβ at a site displaced from the binding interface for Runx1, that is, these compounds function as allosteric inhibitors of this protein-protein interaction, a potentially generalizable approach. Treatment of the human leukemia cell line ME-1 with these compounds shows decreased proliferation, indicating these are good candidates for further development.

INTRODUCTION

The protein-protein interaction between the subunits of the heterodimeric transcription factor CBF, core binding factor β (CBFβ), and Runx1 (CBFα) plays a critical role in hematopoiesis (blood cell development) [1]. CBFβ functions to increase the DNA binding of the Runx1 subunit 20- to 40-fold [2] and to protect the Runx1 subunit against ubiquitination and subsequent proteasome degradation [3]. The gene coding for the CBFβ subunit (*CBFB*) is the target of a common chromosomal translocation, inv(16), found in 12%–15% of acute myeloid leukemia cases [4]. This translocation results in the fusion of the N-terminal 165 amino acids of CBFβ to the coiled-coil region of smooth muscle myosin protein (hereafter referred to as CBFβ-SMMHC). The CBFβ-SMMHC fusion protein causes dysregulation of CBF function [5] in part by means of anomalously tight binding to Runx1 [6]. Because binding to Runx1 is required for the dysfunction associated with this protein, this binding represents an excellent target for inhibition as a potential therapeutic strategy. A small-molecule inhibitor of this kind has the potential to be a useful and highly specific therapeutic agent. We have developed small molecules which bind to CBFβ and inhibit Runx1 binding. This represents the first step toward our overall goal of developing compounds which can specifically inhibit CBFβ-SMMHC while minimally perturbing the activity of CBFβ itself.

Although there is a long history of development of small-molecule inhibitors of enzymes by means of targeting their active sites, the development of inhibitors of protein-protein interactions has been relatively limited [7–10]. Potential difficulties in developing inhibitors of protein-protein interactions include the large surface area typically buried at such interfaces, the lack of significant curvature on these interfaces [7], and the increased mobility of the side chains and often the backbone at these interfaces [11, 12]. Recent successes in the development of small-molecule inhibitors of protein-protein interactions [7–10, 13–17] suggest these barriers are surmountable. Mutagenesis studies of protein interfaces have clearly demonstrated that only a small subset of amino acids at a particular interface contributes the vast majority of the binding energy. These results clearly imply that targeting of molecules to these so-called hot spots is highly likely to disrupt the interaction and that extensive coverage of the protein interface with relatively large molecules is not required. Indeed, recent success in the development of protein-protein inhibitors targeting Rac1 [13] and Bcl-2 [14] bodes well for structure-based approaches to this. An intriguing

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alternative approach is the development of allosteric inhibitors [18] of protein-protein interactions in which the molecule would bind at a site offset from the protein-protein interface and thereby not have to compete with the binding partner. Indeed, examples of such allosteric inhibitors have been demonstrated for β-lactamase [19], LFA-1 [20], and nitric oxide synthase [21], suggesting this may be a generalizable approach.

We have solved the 3D structure of CBFβ using solution NMR [22] and mapped the binding interface with Runx1 by both chemical shift perturbation as well as Ala mutagenesis of the binding interface [23]. This provided the framework to carry out virtual screening, and subsequent screening by chemical shift perturbation in 2D ^{15}N-^{1}H HSQC spectra of CBFβ to identify potential lead compounds. A FRET-based assay and an ELISA have been developed to measure the effectiveness of these compounds in inhibiting the CBFβ-Runx1 Runt domain interaction. Using a traditional medicinal chemistry approach, we have elaborated these compounds to identify structure-activity relationships, resulting in compounds with significant inhibitory potency (low micromolar range). We have used the NMR chemical shift perturbations to dock the compounds to CBFβ and show that they bind at a site offset from the interfacial region of CBFβ. These data show that they act in an allosteric manner to inhibit the binding of Runx1 to CBFβ. These inhibitors demonstrate activity in a cellular FRET assay in HEK293 cells and inhibition of proliferation of the leukemia cell line ME-1 (containing the inv[16]), indicating they are good leads for further development.

RESULTS

Lead Identification and NMR Chemical Shift Perturbation Screening

The computer program LUDI/InsightII (Accelrys) [24, 25] has been utilized for virtual screening using the Available Chemicals Directory database of ~70,000 commercially available compounds with drug-like properties. The 20 conformers of CBFβ used to represent the solution structure of the protein were employed for virtual screening, which resulted in 35 compounds selected for experimental screening using NMR spectroscopy. Screening by means of observation of chemical shift changes in the 2D ^{15}N-^{1}H or ^{13}C-^{1}H HSQC spectra of proteins in the presence of ligands has been very successfully applied [26–29]. In this study, we have employed chemical shift perturbations in the 2D ^{15}N-^{1}H HSQC spectra of CBFβ in the presence of ligands to identify compounds that bind to the protein as well as to provide information on the location of their binding site. Four of the 35 compounds showed significant chemical shift changes in 2D ^{15}N-^{1}H HSQC spectra of CBFβ. Data for one of the four are shown in Figure 1A. The structures of these four molecules (Table 1, compounds **1–4**) share a common substructure, namely a 2-aminothiazole attached to an aromatic ring. We also titrated CBFβ with each of the initial compounds to get an estimate of the binding constant, which showed they

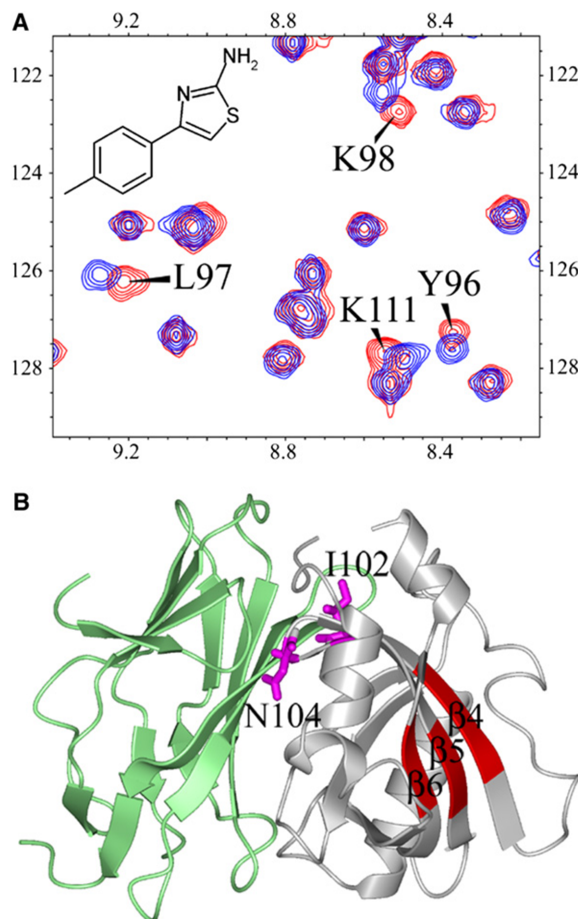

Figure 1. Interactions of 2 with CBFβ

(A) Selected region of the ^{15}N-^{1}H HSQC spectrum of CBFβ (red) and CBFβ plus **2** (blue). Amides perturbed upon binding are labeled.

(B) Residues with chemical shift perturbations (red) mapped onto the structure of CBFβ (gray) complexed to the Runx1 Runt domain (green). The side chains of N104 and I102 are shown in magenta.

all bind with low millimolar dissociation constants. Because we have previously assigned the NMR spectra of CBFβ, these data also provide an indication of the binding site on CBFβ. Figure 1 shows the chemical shift perturbations observed for one of the lead compounds mapped onto the structure of CBFβ. Interestingly, the observed perturbations are consistent with binding at a site displaced from the Runx1 interface (Figure 1B). Because these lead compounds are not binding directly at the interface, any inhibitory effects of these compounds would necessarily have to occur by means of an allosteric or noncompetitive mechanism.

FRET Assay

In order to assess the effectiveness of these compounds in inhibiting binding of the Runx1 Runt domain to CBFβ, we have developed a FRET assay to measure this binding (Figures 2A and 2B). We fused the green fluorescent protein derivative Cerulean to the N terminus of the Runt

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