

Short Conceptual Overview

Paradoxical inhibition of cellular protein expression by proteasome inhibitors

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Abstract

Proteasome inhibitors are used as anticancer drugs, however, the precise mechanisms of their selective activity against cancer cells are not understood well. While proteasome inhibitors stabilize the majority of cellular proteins through inhibition of proteasome activity, they also paradoxically downregulate several other proteins. We recently discovered that proteasome inhibitors suppress mRNA and protein expression of FOXM1, NPM, and ARF proteins that are involved in cancer. We postulated that proteasome inhibitors preferentially stabilize negative regulators of transcription of these genes, which overrides their protein stabilization. These data suggest a presence of multiple secondary mechanisms that may regulate transcription, degradation, or localization of cellular proteins after treatment with proteasome inhibitors. Future experiments will identify these mechanisms and additional proteins suppressed by proteasome inhibitors, and will help explain the role of protein suppression by proteasome inhibitors in their anticancer activity.

Keywords: ARF; FOXM1; negative regulator; NPM; transcription.

Introduction

The 26S proteasome is a multi-subunit protease complex that degrades a wide range of proteins that are tagged with ubiquitin conjugates (1). Ubiquitin, a major element of the ubiquitin-proteasome system, is composed of a highly conserved 76-amino-acid sequence, which is covalently attached by ubiquitin ligases to cellular proteins and targets them for degradation by the proteasome. The 26S proteasome includes two 19S regulatory particles and one 20S core particle, which mediates the process of indiscriminate destruction of cellular proteins (2). Inhibition of proteasome activity influences a broad range of cellular processes, and has been shown to be efficient for anticancer therapy. Proteasome inhibitors (PIs) were developed as anticancer drug candidates from experiments that have shown that PIs can induce specifically cell

death in leukemia- and lymphoma-derived cancer, but not in normal cells (3). However, the exact mechanism of the anticancer activity of PIs is still elusive. As the proteasome mediates proteolytic degradation of the majority of cellular proteins, and PIs hinder this activity, an increase of protein expression is a hallmark of PIs. However, contrary to this, we identified several cellular proteins that are suppressed by PIs. In this overview, I will discuss this paradoxical effect and its importance for cancer treatment.

Negative regulation of FOXM1

Forkhead box (Fox) M1, FOXM1, is a transcription factor of the Forkhead family that induces the expression of genes involved in cell cycle progression and is required for the execution of the mitotic program (4). *FOXM1* is one of the most overexpressed genes in human solid tumors (5, 6), while its expression is low in normal cells (4). We decided to identify inhibitors of FOXM1 transcriptional activity as potential anticancer drugs by screening of a cell-based system using national cancer institute (NCI) libraries of small molecules (7). As a result of the screening, we identified the thiopeptide siomycin A (NSC-285116), which acts as an inhibitor of FOXM1 transcriptional activity and FOXM1 expression (7). Later, another thiazole antibiotic, thiostrepton, structurally similar to siomycin A, was also identified as a FOXM1 inhibitor (8, 9). In addition, we demonstrated that only siomycin A and thiostrepton (8) because of their specific structures (9), but not other known thiazole antibiotics, such as berninamycin, micrococcin P1 and P2, thiocillin, and YM-266183, act as PIs and suppress FOXM1. The link between proteasome inhibition and suppression of FOXM1 established for siomycin A and thiostrepton suggested that bona fide, well-known PIs might also inhibit FOXM1. Therefore, we investigated how PIs affect FOXM1 transcriptional activity and protein expression. Indeed, we found that the PIs MG115, MG132, and bortezomib inhibit FOXM1 transcriptional activity and repress FOXM1 protein expression (10), because FOXM1 is involved in a positive autoregulation loop and it activates its own transcription (11).

On the basis of these observations, we proposed the following hypothetical model of negative regulation of FOXM1 by PIs: proteasome inhibition leads to an increase of the expression of a hypothetical negative regulator of FOXM1 (NRFM) that inhibits transcriptional activation of FOXM1 by FOXM1 (12). As a consequence of the inhibition of FOXM1 transcription by NRFM, PIs suppress the expression of both FOXM1

mRNA and protein (10). As protein stabilization of NRFM by PIs is a common feature of PIs, it may explain the general mechanism of FOXM1 suppression by PIs. Therefore, generally speaking, PIs should inhibit FOXM1 independently of their structures and FOXM1 represents a universal target of PIs. As FOXM1 might be the Achilles' heel of cancer (13), we proposed that FOXM1 is a main target of PIs in cancer cells (12). To identify the NRFM, we developed a cell line in which we introduced doxycycline-inducible FOXM1 that transcriptionally upregulates the expression of the gene that gives resistance to geneticin. These cells died in the presence of geneticin, but survived on geneticin in the presence of doxycycline. However, when we introduced PIs, thiostrepton or bortezomib, cells died (unpublished data). We propose that PIs stabilized the NRFM that inhibits FOXM transcriptional activation that conferred resistance to geneticin. We plan to rescue these cells by targeting of NRFM by shRNA from a pooled shRNA library. Analysis of surviving cells will help identify shRNA that targets NRFM and establish the role of NRFM in the suppression of FOXM1 by PIs.

Negative regulation of NPM and ARF

The phosphoprotein chaperone nucleophosmin (NPM) (14, 15) and the tumor suppressor ARF (16) are involved in cell proliferation, programmed cell death, and oncogenesis (16, 17). In addition, both proteins have been shown to interact with FOXM1 and with each other, and have opposite functions (18–20). While ARF inhibits FOXM1 function and loss of ARF accelerates FOXM1-induced tumorigenesis (18), NPM expression is required for FOXM1 stability and activity (20). We decided to test how different PIs may affect NPM and ARF expression (21). We treated human cancer cells with PIs (thiostrepton, MG132, and bortezomib), and the levels of ARF and NPM were measured by quantitative reverse transcription-PCR and immunoblotting. Following individual treatments with thiostrepton, MG132, and bortezomib, the levels of ARF and NPM mRNA were suppressed from 30% to 80%, while the levels of proteins were suppressed by 20–80% (21). The mechanisms that may explain the inhibition of ARF and NPM by PIs are not known.

Similarly to the regulation of FOXM1 by NRFM, we proposed that PIs stabilize negative regulators of the transcription of these genes because PIs inhibit their transcription (21). The suppression of the transcription of these genes leads to the inhibition of ARF and NPM protein expression, and reverses the stabilization of these proteins by PIs. Interestingly, FOXM1, NPM, and ARF proteins are targets of the ubiquitin-proteasome system and when FOXM1 was expressed from an exogenous promoter, FOXM1 protein was stabilized by PIs. This result suggests that secondary/transcriptional regulation is responsible for the suppression of these proteins by PIs. The transcription factor nuclear factor- κ B (NF- κ B), which is responsible for a variety of inflammatory responses, is also negatively regulated by PIs. PIs suppress the NF- κ B transcriptional activity (but not the amount) through stabilization of its partner I κ B and sequestering of NF- κ B in the cytoplasm

where it is not active (22). These examples suggest that preferential stabilization of negative regulators is responsible for the suppression of expression or activity of different cellular proteins by PIs. Further experiments are needed to determine the precise mechanism of inhibition of FOXM1, ARF, and NPM by PIs, and to identify potential transcriptional inhibitors of these genes that are stabilized by PIs.

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