Abstract # 3797

Hyperphosphorylation and Desumoylation of RanGAP1+SUMO1

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Introduction

Rigosertib Sodium (ON 01910.1Na)
Rigosertib is a multi-targeted, oral, small molecule kinase inhibitor that inhibits both kinases and phosphatases [1]. Rigosertib induced hyperphosphorylation of RanGAP1+SUMO1 in all tested cell lines [2]. The inhibition of the CDK1 kinase leads to hyperphosphorylated cyclin D1 in mantle cell lymphoma cells [3]. Rigosertib inhibits a range of serine/threonine kinases through a single, dose-dependent mechanism. This mechanism involves both desumoylation and dephosphorylation.

Materials and Methods

Cell lines: Caco-2, HCT116, HeLa, MCF-7, and DU145. The cells were maintained in RPMI 1640 supplemented with 10% FBS and antibiotics (penicillin/streptomycin). Cells were seeded 24 h before use and treated with 1 µM rigosertib for 24 h. The MTT assay was used to evaluate cell viability.

Western Blot analysis of total cell lysates derived from RIGO-treated cells and subjected to in vitro dephosphorylation reaction with Lambda Protein Phosphatase (PPase). See M&M for details.

Summary

1) Exposure of tumor cells to anticancer inhibitor rigosertib results in cell cycle arrest at mitosis, which is correlated with expression of Hyperphosphorylated RanGAP1+SUMO1 (HRGS). Previously, we suggested inhibition of a putative HRGS phosphatase as the mechanism of action. HRGS becomes easily deSUMOylated under non-denaturing lysis conditions [making it difficult to study this protein in vitro].

2) N-ethylmaleimide (NEM), added to non-denaturing lysis buffer, inhibits deSUMOylation of HRGS in a dose–dependent manner.

3) RanGAP1’s hyperphosphorylation is stable under various lysis conditions, regardless of its SUMOylation status.

4) HRGS dephosphorylation without deSUMOylation is possible with the use of an external phosphatase. This suggests an in vitro test system to identify the responsible enzyme that might be the target of inhibition by rigosertib.

References:


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