

Mechanism of mitotic arrest induced by dolastatin 15 involves loss of tension across kinetochore pairs

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Abstract Dolastatin 15 (DL15) is a potent, tubulin-targeted, vinca-site binding, anticancer agent that induces mitotic arrest and inhibit cell proliferation in a variety of cell types. Several analogs of DL15, including LU 103793 and tasidotin, have been progressed to clinical trials for different types of cancer. DL15 has been known to interfere with cellular microtubules and purified tubulin in vitro. However, the molecular mechanism with which the peptide arrests cells in mitosis is poorly understood. This study reports a possible antimitotic mechanism of action of DL15. DL15 inhibited HeLa cell proliferation in a concentration-dependent manner with a half-maximal inhibitory concentration (IC_{50}) of 2.8 ± 0.3 nM, induced mitotic arrest, disrupted cellular microtubules near its IC_{50} for cell proliferation, and inhibited the re-polymerization of cellular microtubules. By staining the centrosomes of DL15-treated cells with anti- γ tubulin antibodies, the study found a significant reduction in interpolar distances in mitotic HeLa cells, indicating a disruption in the normal assembly dynamics of the microtubules. The study further found that DL15 induced a loss of tension across the kinetochore pairs as indicated by a reduction in interkinetochore distance. In response to this loss of tension, the tension-sensing checkpoint protein BuBR1 accumulated at the kinetochores, promoting mitotic arrest. In vitro, DL15 promoted formation of curved and

fragmented polymers of microtubule proteins and inhibited tubulin decay in a manner similar to vinca-site binding agents such as phomopsin A. Together, the data indicate that the mitotic arrest induced by DL15 involves a loss of tension across the kinetochore pairs due to disruption of normal assembly dynamics of microtubules.

Keywords Cancer · Microtubules · Tubulin · HeLa cells · Kinetochores · Dolastatin 15 (DL15)

Introduction

The optimal regulation of cell division is integral to human health; deficiencies in the checkpoint-mediated control of cell division often lead to hyper-proliferative disorders [1, 2]. Microtubules, which are the dynamic, polarized, linear, cytoskeletal polymers assembled from α - β tubulin heterodimers, play crucial roles in cell division, during which the dynamic microtubules “search and capture” the condensed chromosomes, align them at the metaphase plate, and segregate the chromosomes into the newly forming daughter cells [3, 4]. Microtubule-targeted agents interfere with the natural assembly dynamics of the microtubules through different molecular mechanisms, arrest the cells in mitosis, and inhibit cell proliferation; many of these agents are approved anticancer drugs for the treatment of different types of cancer [5, 6].

Dolastatin, a linear, natural peptide, was originally discovered in the Indian Ocean sea hare, *Dolabella auricularia* [7]. A number of dolastatin analogs have been shown to interfere with microtubules and induce mitotic block [8], and several analogs of Dolastatin 15 (DL15) have shown significant regression of tumors in clinical settings [9–12]. Biochemical and molecular modeling studies have

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