ELSEVIER

Haspin: a promising target for the design of inhibitors as potent anticancer drugs

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Protein kinases constitute a large group of enzymes in eukaryotes and have an important role in many cellular processes. Several of these proteins are active kinases, such as haploid germ cell-specific nuclear protein kinase (Haspin), an atypical eukaryotic protein kinase that lacks sequence similarity with other eukaryotic protein kinases. Haspin is a serine/threonine kinase that associates with chromosome and phosphorylates threonine 3 of histone 3 during mitosis. Haspin overexpression or deletion results in defective mitosis. It has been shown that Haspin inhibitors have potent anti-tumoral effects. Given that the only Haspin substrate is threonine 3 of histone 3, inhibition of Haspin might have fewer adverse Q2 **effects compared with XXXX. Here, we highlight the chemical structures and actions of currently known Haspin inhibitors.**

Introduction

The protein kinases constitute a large group of enzymes in eukaryotes. These enzymes catalyse the transfer of the γ-phosphate of ATP (or GTP) to generate phosphate monoesters using the hydroxyl groups of many proteins. Depending on the proteins involved, this reaction results in either serine/threonine kinases or tyrosine kinases. These protein kinases are related by virtue of their homologous kinase domains and have important roles in many cellular processes (e.g., proliferation, gene expression, metabolism, motility, membrane transport, apoptosis, etc.); unsurprisingly, their misregulation often results in disease. Most eukaryotic protein kinases are members of the eukaryotic protein kinase superfamily. The kinase domain of protein kinases (250–300 amino acids) contains 12 conserved subdomains. The crystal structure of eukaryotic protein kinases shows that they have a bilobed structure (the smaller *N*-terminal lobe and the larger C-terminal lobe). The deep cleft between the two lobes is recognised as the site of catalysis. Three functions are attributed to the kinase domain: (i) binding and orientation of the ATP (or GTP) phosphate donor as a complex with the divalent cation; (ii) binding and orientation of the protein substrate; and (iii) transfer of the γ-phosphate from the ATP to the hydroxyl residue of the protein substrate [1,2]. In 1995, Hanks and Hunter proposed the first classification of eukaryotic protein kinases, based on their structural and functional properties [2]. In 2002, the classification was refined by Manning *et al*. based on the extracatalytic sequence similarity and biological function [3]. Several protein kinases lack sequence similarity with eukaryotic protein kinases and, thus, have been classified as inactive pseudokinases [3].

Haspin (encoded by germ cell-specific gene 2: *Gsg2*) is found in many eukaryotic lineages (animal, fungi, and plants), and the protein and its mRNA were initially detected in male germ cells [4]. All proliferating cell lines express Haspin mRNA, and it has been detected in thymus, bone marrow, and foetal liver and, more weakly, in spleen, intestine, lung, and a variety of fetal tissues. Its expression is correlated with tissues that have

Haploid germ cell-specific nuclear protein kinase (Haspin) proteins are divergent members of the eukaryotic protein kinase family. Q4

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GLOSSARY

Budding uninhibited by benzimidazole 1 (Bub1) and budding uninhibited by benzimidazole-related 1 kinases (BubR1): serine/threonine checkpoint kinases with important roles as kinetochore-microtubule attachment regulators.
Centromere protein A (CENP-A): a centromere-interacting protein (along with CENP-B, CENP-C1, CENP-D, CENP-E and CENP-F).
CDC2-like kinase 1 (CLK1): has an important role in the regulation of RNA splicing through phosphorylation of members of the serine and arginine-rich (SR) family of splicing factors.
Chromosomal passenger complex (CPC): comprises Aurora B, INCENP, Borealin, and Survivin; a key orchestrator protein of orderly mitotic exit and cytokinesis.
DYRK(1A, 1B, 2, 3): members of the dual specificity tyrosine-phosphorylation-regulated kinase family.
Highly expressed protein in cancer 1 (Hec1): located at the centromere during cell mitosis; has an important role in the spindle checkpoint pathway.
Protein kinase Ac (PKAc): catalytic subunit of cAMP-dependent protein kinase.
Repo-Man: nuclear protein that is a specific regulatory subunit for PP1γ. Repo-Man disperses in the cytoplasm as cells enter prophase but relocalises to chromatin at anaphase onset. Repo-Man was identified as a complex responsible for inactivation of a regulator of chromosome architecture in anaphase.
Spindle assembly checkpoint (SAC): sometimes referred to as the 'mitotic checkpoint' or 'M-phase checkpoint'; a quality-control mechanism that prevents anaphase until all the chromosomes are stably attached to the spindle.

significant levels of cellular proliferation and differentiation [5]. Studies have confirmed Haspin as a serine/threonine kinase and a constitutively active enzyme.

Studies have shown that, during interphase, Haspin is autoinhibited by a conserved segment of basic residues (the Haspin basic inhibitory segment; HBIS) within the N-terminal domain, immediately upstream of the kinase domain. The kinase is reactivated in M phase by Cdk1 phosphorylation of the N terminus (Fig. 1). This phosphorylation leads to recruitment of Polo-like kinase-1 (Plk-1), which in turn further phosphorylates multiple sites at the N-terminal domain of Haspin. In addition, in human cells, the localisation of Aurora B kinase to the centromere creates a positive feedback loop that increases Haspin activity [6].

The crystal structure of Haspin reveals at least four peculiarities [5]: (i) the N-terminal lobe is entirely buried under an additional layer created by an N-terminal extension and two insertions; (ii) reorganisation of the activation segment contributes to the creation of an unusual substrate-binding site; (iii) an additional insertion between the β7 and β8 loop that contains two β-strands; and (iv) deletion of the αG helix.

During mitosis, Haspin localises predominantly to condensed chromosomes during mitosis, to centrosomes following nuclear envelope breakdown (NEBD), to spindle microtubules during metaphase, and to the midbody during telophase. The only know substrate of Haspin is histone H3. During mitosis, Haspin phosphorylates histone H3 at threonine 3 to form H3T3 ph [7]. Phosphorylated threonine 3 is detected on condensing chromosomes during prophase, prometaphase, and metaphase, is decreased during anaphase and is absent during telophase. Histone H3T3 ph creates a chromatin-binding site for survivin and recruits the **chromosomal passenger complex** (CPC; see Glossary) at inner centromeres during mitosis [8–12]. Haspin activity facilities the activation of Aurora B, a member of the CPC (Fig. 2). Aurora B kinase activity is necessary for full phosphorylation of Haspin during mitosis and stimulates H3T3 phosphorylation. It also acts to generate a positive feedback between Haspin and Aurora B and allows CPC accumulation on chromatin during mitosis [13]. The complex protein phosphatase 1γ(PP1γ)/**Repo-Man** induces indirect inhibition of XXXX by dephosphorylating of H3T3 at the end of mitosis [14,15]. Q5

Haspin overexpression or deletion results in defective mitosis. Inhibition of Haspin prevents normal chromosome alignment at metaphase, while Haspin overexpression results in a delay before metaphase. In addition, Haspin depletion leads to the loss of the cohesion association and activation of the spindle checkpoint, arresting mitosis in a prometaphase-like state [16].

Haspin inhibitors have antimitotic effects and might have fewer adverse effects than XXXXX because Haspin has only one known substrate (Thr 3 of histone 3) [17]. A previous study showed that Haspin inhibitors cause the displacement of Aurora B from inner centromeres, resulting in its diffuse distribution on chromatin [17]. Therefore, XXXXXXX. Q6

Haspin inhibitors

Acridine derivatives

Using high-throughput screening (HTS), the compound LDN-192960, an acridine derivative, was identified as a potent kinase Haspin inhibitor with IC_{50} = 0.01 MM (Fig. 3) [18]. Based on the structure–activity relationships (SAR) of the acridine series, Cuny *et al.* [19] identified many Haspin inhibitors, the general structure of which is given in Fig. 3. All compounds showed good inhibitory activity again Haspin, while compound LDN-209929 was very selective for Haspin (180-fold selectivity versus **DYRK2**). LDN-209929 was reported to compromise the spindle checkpoint during mitosis when microtubules are severely disrupted [17]. However, the adverse effects highlighted with either acridine or tacrine, for example, do not support the use of this scaffold in the development of drugs for use in humans.

Beta carboline derivatives

Harmine and harmalol

Harmine is an indole alkaloid from the plant *Peganum harmala* with the pyrido[3,4-b]indole ring structure characteristic of β-carboline family alkaloids (Fig. 3), which includes harmaline, harman, and harmalol. These compounds exhibit psychoactive activity on the central nervous system (CNS), with hallucinogen adverse effects. Harmine has also been identified as an inhibitor of DYRK family kinases, with IC_{50} values of 0.03–0.35 MM reported for DYRK1A, and approximately 50-fold lower potency toward DYRK2 [20–23]. More recently, using time-resolved fluorescence resonance energy transfer (TR-FRET), Cuny *et al.* showed harmine and harmalol to be moderately potent Haspin inhibitors, with IC_{50} values of 0.59 and 0.77 MM, respectively [24]. The adverse effects

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