

Understanding HAIs: Ally proteins in the fight against cancer

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Keywords

ally protein; cancer; chaperone; HAI-1
HAI-2; malignant tumour; matriptase

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(Received 2 February 2022, revised 25
February 2022, accepted 15 February 2022)

doi:10.1111/febs.16399

Understanding how HAI-1 and HAI-2 regulate the epithelial serine protease matriptase may hold the key to curing epithelial-derived cancer. HAIs are serine protease inhibitors that inhibit matriptase and have a poorly understood effect on the presence of matriptase protein in cells. In this issue of *The FEBS Journal*, Yamashita *et al.* provide much-needed new insights into this effect, describing it as a ‘chaperone-like function’ of HAI-1. However, several observations suggest that matriptase folds correctly without HAIs and that HAIs are not chaperones. We introduce the concept of ‘ally proteins’ to categorize the poorly understood function of HAIs, distinguishing them from chaperones.

Comment on: <https://doi.org/10.1111/febs.16348>

Introducing matriptase: A target for cancer therapy

Ninety percent of cancers today are believed to be of epithelial origin. The matriptase pathway is essential for epithelial homeostasis and dysregulation causes epithelial-derived cancer, making it a potential cancer therapy target.

Matriptase is a membrane-anchored serine protease that proteolytically activates at least two pro-carcinogenic signal transduction pathways [1,2]. Mouse studies have shown that uncontrolled matriptase activity causes a high incidence of cancer. This was first seen in transgenic mice overexpressing matriptase under the keratin-5 promoter, which led to dysplasia in 10 out of 10 mice, of which seven became malignant within 2 years [3]. However, matriptase overexpressed together with the serine protease inhibitor HAI-1 led to almost normal cancer incidence [3]. Similar results were later obtained using the HAI-1 homologue HAI-2, suggesting

that re-introduction of a matriptase inhibitor causes regression and complete remission of malignant tumours [4]. This underlines the importance of HAIs in matriptase regulation and matriptase-associated cancer development. Improving our understanding of the interplay between matriptase and HAIs could be the key to curing malignant epithelial tumours.

HAIs: More than just protease inhibitors

Matriptase is first synthesized in an unusual proteolytically active zymogen form (proenzyme) that must be cleaved in its serine protease domain (SPD) to obtain its full activity.

Matriptase activation can be catalysed through auto-activation by active zymogen matriptase or activated matriptase [5]. Matriptase can also be activated by another serine protease, named prostasin. Prostasin

Abbreviation

HAI, hepatocyte growth factor activator inhibitor.

can activate matriptase in both its active zymogen form and its activated form [6]. The four so far identified catalysers of matriptase activation can all be inhibited by HAIs [5,6] (Fig. 1). However, it is becoming increasingly evident that HAIs are more than just inhibitors of serine proteases.

Many research groups have observed that matriptase protein is undetectable while mRNA is present in cells transfected with matriptase, but not HAIs. Conversely, cells co-transfected with both matriptase and HAIs contain high levels of matriptase mRNA, as well as detectable protein [7,8]. The mechanism behind this phenomenon is rarely discussed, as the HAIs are almost always present when matriptase is studied by recombinant expression in cells.

To explain the poorly understood connection between HAIs and the presence of matriptase protein in cells, HAIs have been proposed to affect cellular transport of matriptase along the secretory pathway [9,10], to chaperone [7,8,11], and to stabilize matriptase protein expression [8]. In the present issue of *The FEBS Journal*, the study by Yamashita, et al. [12] successfully gives us new insight into this phenomenon by describing a ‘chaperone-like function’ of HAI-1.

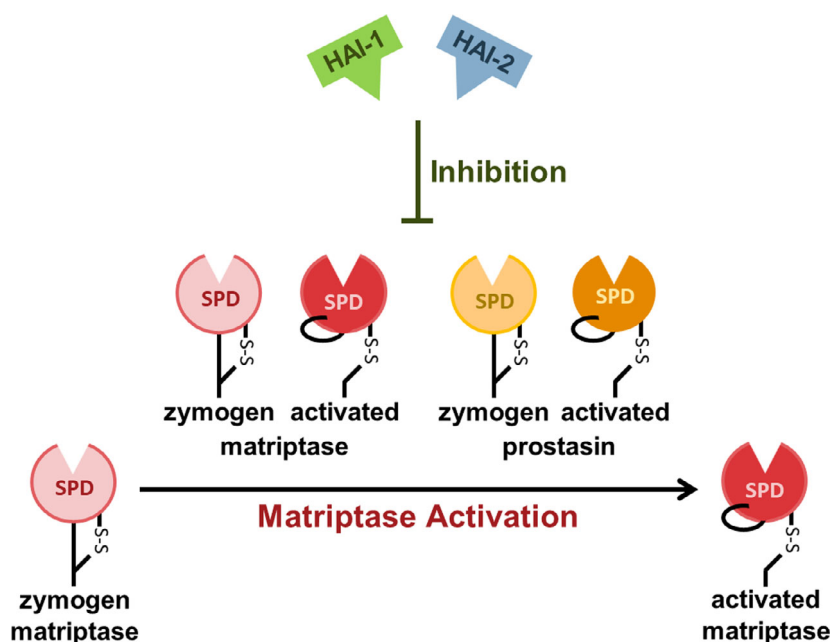
HAIs are essential ally proteins – not chaperones

Although HAIs appear to influence the presence of matriptase protein in cells, several observations suggest that matriptase does not require a chaperone.

Chaperones in the endoplasmic reticulum (ER) are proteins that assist and retain immature proteins until they have obtained correct folding, and thereby control protein transport along the secretory pathway. If correct folding is not obtained, misfolded proteins typically retro-translocate across the ER membrane, where they undergo proteasomal degradation in the cytosol. However, inhibition of proteasomal degradation with the proteasome inhibitor MG132 does not appear to affect the presence of matriptase protein without HAIs in HEK293 cells (A. W. Nonboe and L. K. Vogel, unpublished). Furthermore, several SNPs and matriptase mutants, for example, matriptase R614A, matriptase G827R and matriptase S805A can be recombinantly expressed [8] and detected on the cell surface without HAIs present (L. K. Vogel et al., unpublished). Transport along the secretory pathway to the plasma membrane suggests that a correct protein fold has been obtained despite mutations. The SPD of matriptase has successfully been denatured, purified from *E. coli*, and refolded without the help of chaperones [5]. Full-length matriptase also appears to fold spontaneously without other proteins, as matriptase added to SDS sample buffer, followed by SDS/PAGE, can be incubated in an appropriate buffer, and shortly thereafter regain enzymatic activity (zymography) [13]. Collectively, these findings indicate that HAIs are not essential for matriptase protein to obtain its correct folding. HAIs are therefore not chaperones, but affect the matriptase protein in an unknown way.

Both matriptase and HAI-1 are known to have several conformational forms [14,15] and the HAIs are

Fig. 1. Schematic illustration of matriptase activation and inhibition. Matriptase is synthesized in a proteolytically active zymogen form (zymogen matriptase) that has to be cleaved in the SPD to obtain its fully activated form (activated matriptase). Activation of zymogen matriptase can be catalysed through auto-activation by another active zymogen matriptase and an activated matriptase or through activation by another serine protease, named prostaticin. Prostaticin can activate matriptase in its active zymogen form (zymogen prostaticin) and its activated form (activated prostaticin). The proteolytic activity of all four identified catalysers of matriptase activation can be inhibited by the serine protease inhibitors HAI-1 and HAI-2.



likely involved in conformational changes of matriptase, described by Yamashita et al. [12] as altered antibody recognition.

We introduce the concept of ‘ally protein’ to categorize and describe the poorly understood effect of HAIs on the presence of matriptase protein in cells, to distinguish them from chaperones. Referring to HAIs as ally proteins will represent a unique new keyword to ease future communication and literature searches on this important subject.

It is not clear whether it is the inhibitory function and/or the ally protein function of the HAIs that prevents or reverses the development of matriptase associated cancers. Research into these matters is necessary and may hold the key to curing epithelial-derived cancer. We thank Yamashita et al. [12] for being bold enough to embark on this journey.

Acknowledgement

The work was supported by the Danish Cancer Society.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

LKV drafted the manuscript. LKV, AWN and ZHB reviewed and edited the manuscript. AWN prepared Figures and all authors approved the final version of the manuscript.

Data availability statement

The unpublished data that support the findings of this commentary are available from the corresponding author, LKV, upon request.

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