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Nuclear asparaginyl endopeptidase activity facilitates aberrant cell cycle progression and cellular division in cancer cells

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Introduction

Lysosomal proteases are overexpressed and associated with poor prognosis in a vast majority of human tumours, and their contribution—through their intralysosomal activities—to cancer progression (*e.g.*, tumour angiogenesis, tumour invasion, etc) is well-described[1-2]. Growing evidence has started to reveal a key role for lysosomal proteases in both the cytosolic and nuclear compartment of cells, both under physiological and pathological conditions (*i.e.,* neurodegenerative diseases and cancer)[3-6]. However, the biological processes regulated by these proteases outside the lysosomal compartment remain largely unexplored. Recent reports and preliminary data generated by us are starting to reveal the targets and biological processes governed by these proteases through their extralysosomal functions, however our incomplete knowledge about their nuclear and cytosolic targets hampers their use as therapeutical targets in cancer. Therefore, a high-throughput approach allowing us to unveil the full array of targets and processes controlled by these proteases, outside the lysosomal compartment, is required to fully harness their potential as therapeutical targets.

Objectives

Among these lysosomal proteases, Asparaginyl Endopeptidase (AEP)—a unique lysosomal protease, related to caspases and separase, and the only known protease specifically hydrolysing asparaginyl peptide bonds[7]—is overexpressed in a vast majority of human solid tumours, correlating with poor prognosis and increased malignancy, and showing nuclear localisation[8-10]. However, its role in cancer onset and progression has not been formally addressed. Thus, we aim to investigate the targets and functions controlled through its extralysosomal activity, as an approach to reveal its role in the nucleus of cancer cells and how it contributes to the onset and progression of cancer.

Methods

We have applied high-throughput mass spectrometry—combining chemical inhibition of AEP together with TurboID approaches—, to identify its potential, novel nuclear targets, in combination with Gene Ontology analysis to unveil the biological processes this protease regulates in the nuclear compartment of cancer cells. Finally, we have resorted to molecular and cellular approaches to further investigate the role that the nuclear activity of this protease plays in cancer biology.

Results

Our combined, high-throughput proteomics data revealed a novel, unexpected role for AEP in the nucleus of cancer cells, by directly controlling the levels of proteins playing critical roles in cell cycle checkpoint, DNA damage response and cell division. Furthermore, AEP inhibition by small molecules (*i.e.*, MVO26630) reduced the proliferation of different cancer cell lines. Interestingly, shRNA-mediated AEP KD showed a more drastic effect, thus confirming our observations that while MVO26630 was able to completely block lysosomal AEP activity, it was only able to partially block its nuclear form, suggesting structural/conformational differences between the lysosomal and nuclear forms of this protease. Finally, shRNA-mediated AEP KD resulted in cell cycle arrest, increased genomic instability and DNA damage response and cell death.

Main Conclusions

In conclusion, we have here identified an unexpected role for AEP in the nuclear compartment of cancer cells, by targeting key proteins involved in cell cycle checkpoints, DNA damage response and mitosis, facilitating aberrant cell cycle progression and cell division in cancer cells, while promoting genomic instability. Importantly, AEP

deficient mice are viable, showing just a minor kidney phenotype and splenomegaly indicating that the critical role played by AEP as a facilitator of cell cycle progression and mitosis in cancer cells is not required for normal cells, thus offering novel, potential therapeutical opportunities for the treatment of cancer. In addition, our data provides information about other key biological processes that potentially could also be regulated by AEP through its extralysosomal functions (*e.g.*, gene expression, histone modification, etc).

References

1. Davidson SM and Vander Heidein M. (2016). Annual Review of Pharmacology and Toxicology, 57: 481.
2. Machado E et al. (2021). Front Cell Dev Biol 9: 642494.
3. Zhang Z et al. (2014). Nature Medicine 20: 1254.
4. Zhang Z et al. (2020). Transl Neurodegeneration 9: 1.
5. Reinheckel T and Tholen M. (2022). FEBS Open Bio 12: 694.
6. Kos J et al. (2022). FEBS Open Bio 12: 708.
7. Chen et al. (1998). FEBS Lettet 441: 361.
8. Edgington LE et al. (2013). JACS 135: 174.
9. Haugen MH et al. (2015). Eur J Cancer 51: 9.
10. Gawenda et al. (2007). Breast Cancer Res Treat. 102: 1.