

# The future of prostate cancer treatment

Dr Hannu Koistinen explains how research into proteases at the University of Helsinki may pave the way for novel diagnostics and therapeutics in the battle against prostate cancer



**What are you setting out to achieve over the course of your research?**

My overall aim is to improve treatment and diagnostic methods for prostate cancer by studying proteases expressed in the prostate.

**Could you outline the limitations associated with current treatments for prostate cancer?**

Prostate cancer, which in Western countries affects about every seventh man, is a considerable healthcare problem. Its incidence is increasing because of the use of opportunistic screening with prostate-specific antigen (PSA), which has led to the detection of cancers that would not have surfaced clinically without screening. This apparent overdiagnosis has in turn led to overtreatment, because there is currently no way to tell which cancers need to be treated and which would not cause symptoms during the lifetime of the patients. Androgen deprivation therapy is widely used for treatment. However, men treated with this approach commonly develop so-called castration-resistant prostate cancer, for which effective enough therapies are not available.

**By what means are you investigating the slow growth of prostate cancer? Do you have any findings to report on this front thus far?**

The lack of suitable models able to address the heterogeneity between different individuals

and between primary tumour and metastatic lesions is resulting in a very high attrition rate – or slow success rate – in cancer drug development. The tumour models we use do not recapitulate the slow growth generally observed in the early stage of prostate cancer. So far, we have performed some limited treatment studies with our molecules, and have sometimes seen reduced tumour growth. But these studies are still ongoing.

**Peptide research is an emerging field of drug discovery. Have advances been made in any areas aside from prostate cancer?**

Our project still has some way to go before curing prostate cancer. Several currently used drugs are peptides or based on peptides. Examples of peptide drugs include Abarelix, a gonadotropin-releasing hormone antagonist for treatment of prostate cancer; the immunosuppressant Cyclosporin-A, which was initially isolated from a fungus; and the HIV fusion inhibitor Enfuvirtide, which is a synthetic peptide mimicking part of HIV-1 fusion machinery. Generally, peptides such as these cannot be administered orally, but there are several ways to circumvent this and other shortcomings of peptides, and more are generated actively. Due to their size, peptides are attractive drug candidates for mechanisms that can't be targeted with traditional small molecule drugs. These mechanisms include targeting of protein-protein interactions.

**In what ways are you aiming to improve the efficacy of the peptides?**

There are many ways to achieve this, but if we ultimately want the peptides to be used for the treatment of patients, the compounds should preferentially be orally available. To this end, we are developing so-called peptidomimetic analogues, in which the peptide is replaced with artificial

chemical structures that mimic the peptide. This involves structural studies, the information from which is used for synthetic modifications of the peptides and for the search of scaffolds for the synthesis of peptidomimetics. In this collaborative project, we have already been able to make pseudopeptides by replacing parts of the peptides with mimetic structures, without losing the bioactivity.

**With whom are you collaborating over the course of the project? What insight and expertise have they brought to the table?**

This project does indeed involve wide collaboration. My major collaborator in the project is Emeritus Professor Ulf-Håkan Stenman, who initiated the peptide development for PSA. The enhancement of the properties of the peptides is especially challenging and requires a lot of different expertise. In this area, my main collaborators are Drs Ale Närvänen, Erik Wallén and Maija Lahtela-Kakkonen; and Professors Kristina Luthman and Antti Poso. Wallén's group is making the primary contribution in the development of peptidomimetics. Currently, myself and Dr Matthias Nees are planning to widen the studies to consider a broader range of proteases. Nees has established several useful cell models at the VTT Technical Research Centre of Finland.



# Promising proteases

By researching proteases expressed in the prostate, a team at the **University of Helsinki** is aiming to improve the quality of life and reduce mortality of prostate cancer patients

**PROSTATE CANCER IS** a significant healthcare problem that requires early diagnosis and judicious treatment in order to prevent mortality. Currently, prostate cancer affects roughly 15 per cent of men in Western countries. The development of novel therapies, as well as the improvement of diagnostic methods, is proving vital for three reasons: to increase patients' lifetime; to ease the side effects of treatment; and to avoid unnecessary treatment. Thus, the call for more reliable screening and prognostic methods, and improved therapies, has never been more imperative. As prostate cancer generally develops less quickly than other cancers, even the smallest abatement in tumour growth could prove significant.

Research being conducted by the Department of Clinical Chemistry at the University of Helsinki is looking at how increased proteolytic activity – which is associated with numerous diseases, including cancer – may have a functional role in prostate cancer growth. The prostate generates several proteases, the most prevalent of which belong to the kallikrein-related peptidases (KLKs): prostate-specific antigen (PSA, KLK3) and glandular kallikrein (hK2, KLK2). KLKs are recognised as potential targets for drug discovery, having garnered much attention in their role as biomarkers. However, our knowledge of the use of KLKs as drug targets remains rudimentary, and there are currently no KLK-based drugs on the market.

Dr Hannu Koistinen is the principal investigator leading the project, examining the function of these proteases, utilising the cell culture and xenograft tumour models associated with prostate cancer. The study aims to identify the mechanisms that can be employed for new methods of therapy, such as the use of compounds that modulate the behaviour of prostatic proteases.

## PROTEASES AND PEPTIDES

The project is developing peptides that bind to PSA or hK2 – either triggering PSA activity or preventing

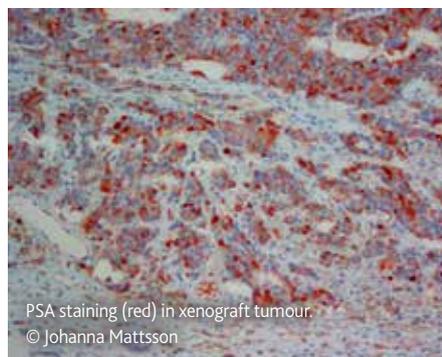
that of hK2. Tumour growth may be reduced, even blocked, by strengthening the antiangiogenic effect of PSA and abating hK2 activity. The hypothesis proposes that modified peptides can then be employed in both imaging and the prevention of prostate cancer growth. Research has shown that PSA reduces angiogenesis, while hK2 is active in proteolytic cascades, which could promote the growth of a tumour.

PSA's antiangiogenic behaviour reduces blood vessel growth. Therefore, stimulating its activity through the utilisation of peptides could hinder tumour growth. Conversely, hK2's tendency to increase tumour growth may also be a factor in the formation of metastases, and so its inhibition by peptides could prove therapeutic. The peptides being developed will help to create drug molecules – an aspect of research that is part of a wider, internationally collaborative project.

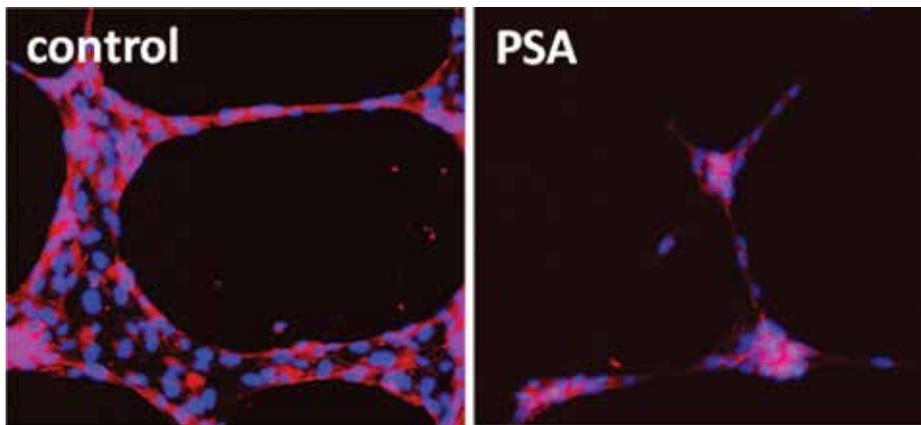
Before establishing his own research group four years ago, Koistinen worked in the group of Professor Ulf-Håkan Stenman, head of the Department of Clinical Chemistry at that time, who was already studying the diagnostic qualities of PSA and had advanced the diagnosis of prostate cancer through the development and validation of clinical assays for different forms of PSA. Explaining the origins of this line of investigation, Koistinen notes: "PSA and its close relative hK2 are the most abundant prostatic proteases, but still their biology is only partially known, especially whether they have any role in prostate cancer development. Proteases are generally involved in cancer and have major role in the formation of metastases, which eventually kill the cancer patients". He and his collaborators hypothesised that PSA and hK2 are involved in prostate cancer development, and they continue to explore the possibility that PSA and hK2 are vital factors in the treatment of prostate cancer. However, Koistinen plans to widen the scope of the project by analysing the roles of other proteases.

## TARGETING MOLECULAR IMAGING

The evidence collected so far, suggesting that PSA and hK2 may be useful targets for non-invasive imaging of prostate cancer metastases, is promising. Both PSA and hK2 have proved very attractive targets for molecular imaging, having been discovered at high levels in prostate and prostate cancer metastases. In order to choose treatments, reliable imaging of metastases is paramount. Due to PSA and hK2 expression being induced by androgens, the two enzymes could also be effective for the imaging of response to androgen-deprivation therapy and relapse, which was recently indicated by researchers working at the Memorial Sloan-Kettering Cancer Center.



PSA staining (red) in xenograft tumour.  
© Johanna Mattsson



PSA reduces tube formation of endothelial cells in cell culture model, indicating reduced angiogenic potential of the PSA treated cells. © Johanna Mattsson

Koistinen suggests that the developed peptides would be viable for imaging, because they only bind to active forms of proteases – those found in tumour tissue, but not in blood. Peptides also disappear from the body quickly, which means imaging can be done in less time than with larger antibodies. One disadvantage of the developed peptides, however, is that their natural binding strength is not as good as that of antibodies.

**Tumour growth may be reduced, even blocked, by strengthening the antiangiogenic effect of PSA and abating hK2 activity**

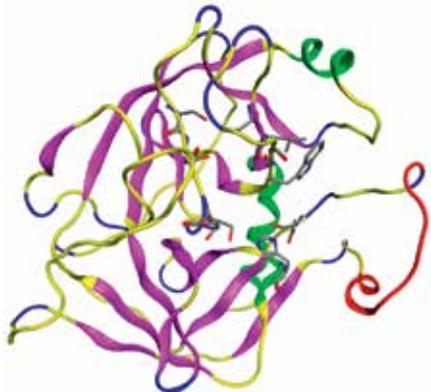
#### IN VIVO MODELS AND THE DEVELOPMENT OF BETTER COMPOUNDS

One of the big obstacles faced by the project is in translating studies of proteases from the laboratory bench to *in vivo* models. As PSA and hK2 are not expressed in mouse, their functions cannot be studied in knock-out models. Some mouse models that express PSA and hK2 artificially – that is, transgenic models – have been developed, but the expression levels of these proteases

are very low compared to those in the human prostate. As such, the group has used mice transplanted with tumours consisting of human cancer cells to study the effects of molecules that modulate the proteolytic activity of PSA and hK2. Peptides were chosen based on their effectiveness as activity modulators, which can then be enhanced further for *in vivo* studies. However, the unstable nature of these activity modulators, coupled with their quick elimination from the body, means their use *in vivo* has been challenging.

Because these peptides are not viable as drug molecules, the project has aimed to create more drug-like molecules that are more conducive to *in vivo* studies. A few methods have been employed, including the modification of peptides, which improves the stability of hK2-inhibiting peptides via cyclisation. The researchers have also been working on the development of nonpeptidic peptide-mimicking compounds to improve the *in vivo* properties, such as stability. In addition, molecular modelling will be used to better understand pharmacophore. This data will be adapted for the development of peptidomimetic compounds, as well as the virtual screening of compounds, which are then tested for their impact on PSA activity. The first active compounds found by virtual screening, and peptides in which part of the peptide structure has been replaced with artificial mimicking structures, have been just reported.

In terms of his foremost achievements to date, Koistinen is unequivocal: “Regarding hK2 and PSA, I would consider running this multidisciplinary project as one of my major achievements. Thanks to my collaborators, first active pseudopeptides have been developed, which allows us now to delve more in depth into biological and treatment studies,” he notes. “Concerning the biological studies performed by my group, the solid demonstration of the dependency of the antiangiogenic function of PSA on its enzymatic activity has been very important for the project.” In the long term, the collaborators remain optimistic about developing novel diagnostics and therapeutics for the treatment of prostate cancer.



Three-dimensional structure of PSA. © Henna Ylikangas

## INTELLIGENCE

### PROSTATIC PROTEASES: FUNCTIONS AND TARGETS FOR TREATMENT OF PROSTATE CANCER

#### OBJECTIVES

- Elucidate functions of prostatic proteases, especially those of PSA and hK2
- Develop drugs targeting these proteases
- Develop diagnostic and prognostic methods for prostate cancer

#### KEY COLLABORATORS

**Professor Emeritus Ulf-Håkan Stenman**, University of Helsinki, Finland

**Dr Erik Wallén**, University of Helsinki, Finland

**Dr Ale Närvänen**, University of Eastern Finland, Kuopio, Finland

**Professor Kristina Luthman**, University of Gothenburg, Sweden

**Professor Antti Poso**, University of Eastern Finland

**Dr Maija Lahtela-Kakkonen**, University of Eastern Finland

**Dr Matthias Nees**, VTT Technical Research Centre of Finland

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**DR HANNU KOISTINEN** is an Academy Research Fellow and Adjunct Professor (Docent) of Biochemistry at the University of Helsinki. He has 85 articles in refereed international scientific journals and is a chair of the Finnish Peptide Society (FIPS). The major aim of his research group, established in 2009, is to elucidate functions of prostatic proteases.



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