SerpinA12 (vaspin) inhibits enzyme Kallikrein 7, which degrades growth factor Midkine in melanoma

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Introduction

SerpinA12 (vaspin) was identified as a new biomarker in melanoma during a phage display screen against metastatic melanoma cells by the Morris lab. The group has since worked on elaborating the role of vaspin and has identified the key cell signalling pathway. Recent *in vivo* findings have supported the role of vaspin in promoting metastasis through a canonical serpin mechanism that involves the blockade of kallikrein 7, which is known to degrade the recently identified pro-metastatic factor, midkine (Fig. 1).

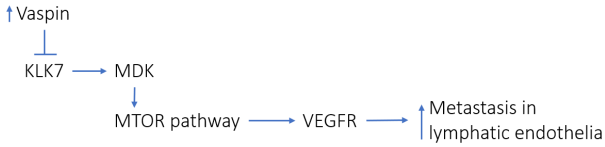

Figure 1. *Hypothesized pathway in melanoma*. Increased levels of vaspin inhibit KLK7, which usually degrades MDK. Non-degraded MDK then induces MTOR pathway, which induces VEGFR. As a result of VEGFR, metastasis in lymphatic endothelia increases.

Aims

1. Confirm that Kallikrein 7 (KLK7) degrades Midkine (MDK);
2. Identify if SerpinA12 (vaspin) inhibits Kallikrein 7 (KLK7);
3. Discover whether the inhibitory serpin activity of vaspin on KLK7 results in the subsequent increases in the levels of pro-metastatic MDK.

Methods

1. *SDS-PAGE* used to test MDK degradation by KLK7 and KLK7 inhibition by vaspin. *Coomassie stain* and *Silver staining* techniques used;
2. *Western blot* used after 1014 melanoma cells transfection in order to overexpress KLK7 to show MDK degradation
3. *Fluorogenic enzyme kinetics assay* used to determine whether KLK7 activation by Thermolysin was successful;
4. *Mammalian cell culture and transfection*;
5. *Bacterial cell culture and plasmid transformation* in order to insert KLK7 and MDK sequences into *E. coli;*
6. *Electrophoresis* used to determine *E. coli* colonies that had the most successful transformation;
7. *BCA assay* used to determine correct protein concentrations of lysed transfected cells.

Outcomes

1. Successfully showed that KLK7 degrades MDK by SDS-PAGE
2. Successfully completed transformation of MDK and KLK7 in *E. coli*
3. Successful activation of KLK7, approved by fluorogenic enzyme kinetics assay
4. Successfully determined protein concentration using BCA assay later on used for SDS-PAGE and Western Blot
5. Maintained mammalian HaCaT and 1014 cells and completed transfection

Results

KLK7 degrades MDK (Fig. 2) but it was challenging to prove vaspin-KLK7 interactions because commercially sourced rh vaspin was undetectable. Complaints were made to the manufacturer and replacements tested. Regrettably, the SDS-PAGE results failed to show a clear result. In the last experiment of the placement, see that vaspin might inhibit KLK7 (Fig. 3), although the quality of vaspin seems to be quite low. While waiting for replacement rhVaspin, I transfected 1014 murine melanoma cells with a pcDNA 3.1 construct overexpressing KLK7 and MDK. The term aim of the experiment was to stable develop lines that overexpress these genes. Agarose gel was used to confirm plasmid DNA integrity before transfection. Western Blotting of KLK7 and MDK was undertaken, but results were not recorded before the end of the placement.

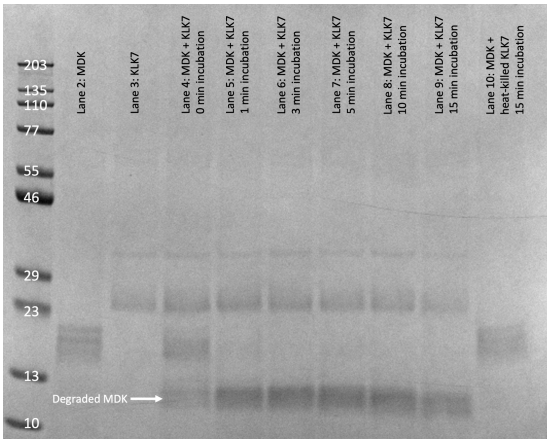

Figure 2. *MDK degradation by KLK7* at different time points from 0 to 15 min at 37 °C shown by SDS-PAGE. Degradation starts at 0 min (lane 4) and is already complete after 3 min (lane 6).

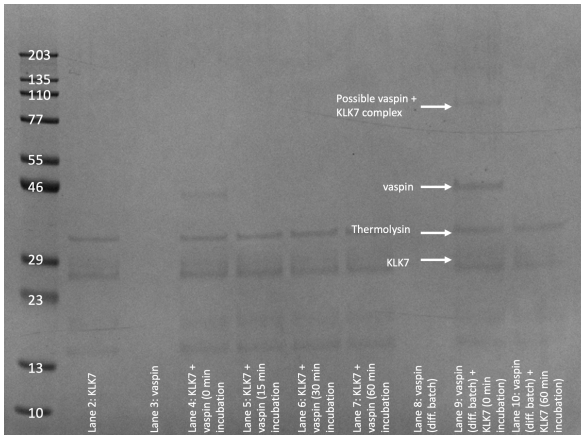

Figure 3. *KLK7 inhibition by vaspin* at different time points from 0 to 60 min at 37 °C shown by SDS-PAGE. Two different batches of vaspin tested: lanes 4-7 and lanes 8-10. In control lanes 3 and 8 no vaspin observed, however in lanes 4 and 9 vaspin is observed. Only in lane 9 there is a faint band of possibly KLK7 and vaspin complex at around 110 kDa.

Future directions

1. Confirming hypothesis of vaspin inhibition on KLK7 using quality vaspin product.
2. Overexpression of MDK, vaspin and KLK7 in melanoma cells and downstream analysis of cell signalling pathways in lymphatic endothelial cells e.g., mTOR pathway

Departures from original project plan

Hypothesis regarding inhibitory activity of vaspin could not be completely evaluated because of the quality of the protein that was delivered. I undertook some *in silico* analysis of the Cancer Genome Atlas, with the aim of identifying RNA expression patterns in clinical melanoma samples.

Value of studentship to the student

1. Laboratory techniques consolidated: SDS-PAGE, Western Blot, Fluorogenic enzymes kinetic assay, handling mammalian cell cultures, bacterial cell cultures, electrophoresis.
2. Data handling skills using The Cancer Genome Atlas (TCGA) Program, looking at mRNA expression regarding vaspin, MDK and KLK7 genes and correlation between these genes.
3. Learned to design experiments, come to my own conclusions, solve arising problems from various experiments troubleshooting.
4. Teamworking skills, as well as data presentation skills during meetings with the supervisor.
5. All of the above mentioned skills are highly beneficial in the career as a scientist and prepared me for my future PhD studies or work in the laboratory in general.

Value of the studentship to the research group

1. Proof of MDK degradation by KLK7.
2. Parathion of MDK/KLK7 plasmids for future transfection studies.
3. Preliminary findings from TCGA data mining.