Proteolytic Cascades of Human Tissue Kallikreins

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ABSTRACT

The human tissue Kallikrein (hK) family is a subgroup of secreted serine proteases, comprised of fifteen members[1]. Recent findings suggest that analogous to other proteases, hKs exert their physiologic and pathologic functions through highly regulated proteolytic cascades [1,2]. In this study, we identified candidate hKs involved in proteolytic cascade(s) activated by hK5. Synthetic heptapeptides (Heps) representing the activation sites of the fifteen hKs were designed. The ability of the active recombinant hK5 to cleave the Heps was examined by reverse phase high performance liquid chromatography (HPLC) and confirmed by mass spectrometry (MS). Cleavage efficiency was quantified as a time-dependent percent reduction of peaks representing uncleaved Heps. Synthetic peptides representing the two cleaved fragments of each Heps were used as controls to eliminate peak reduction due to non-specific degradation. We confirmed the previously reported hK3 cleavage by hK5[3]. Additionally, we identified cleavage of Heps representing hKs 1, 2, 3, 5, 7, 8, 9,11, and 15 by hK5. Activation of pro-hK3 was further confirmed using the hK3-specific substrate,RPY-pNA. We further showed that active hk5 internally cleaves and deactivates excess pro-hK2 and hK3 in a dose-dependent manner. These results indicate that hK5 is involved in a bidirectional regulation of hK2, hK3. Experiments to elucidate the dynamic process of hK proteolytic cascades and their complex tumor-associated dysregulation are ongoing. Therapeutic manipulation of such regulatory pathways of hKs can further be utilized to supplement the current clinical treatments of certain carcinomas.

BACKGROUND

- The hK family is a subgroup of secreted serine proteases, comprised of fifteen members[1].
- Proteases are responsible for hydrolysis of peptide bonds and protein disassembly[4].
- Perturbed protease activity has been shown to contribute to tumorigenesis and metastasis via aberrant extracellular matrix (ECM) remodeling, as well as targeting non-ECM proteins of tumor microenvironment[4].
- Proteases are commonly activated through highly orchestrated proteolytic cascades, e.g. coagulation, caspase-mediated apoptotic, and complement cascades[4].

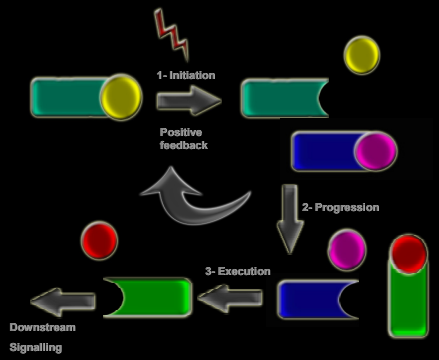


Figure 1: Schematic presentation of proteolytic cascades. Cascade activation is triggered by an external stimuli, e.g. infection, DNA damage, and injury. The initiator protease autoactivates upon stimulation. Active initiator in turn activates downstream protease(s) during the progression phase. These activated proteases are able to activate more of the initiator proteases through positive feedback loops and simultaneously activate their downstream proteases during the execution phase. Active executers elicit proper downstream signals. The main advantage of proteolytic cascades is the rapid response to often minute stimuli [1].

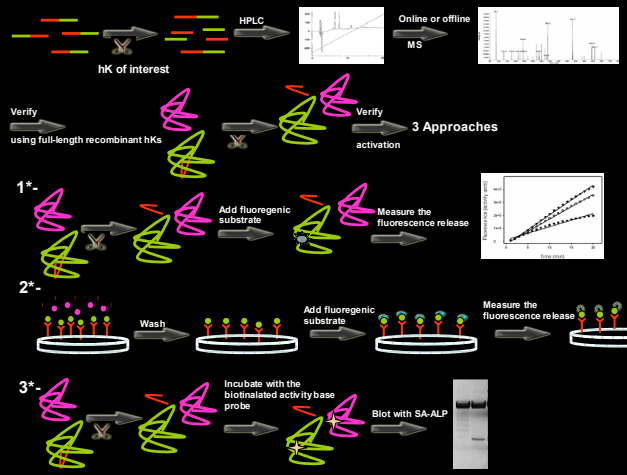
- There are several lines of evidence that certain hKs are activated by other hKs [1,2,3].
- Pro-hKs are activated by cleavage at their preferred trypsin-like cleavage site[1], suggesting that chymotrypsin-like hKs require trypsin-like hKs for activation.
- Certain hKs are co-expressed and confer a common pattern of up- or down-regulation [1].
- Majority of hKs exhibit a common pattern of hormonal regulation[1].

❖ **Therefore, hKs are hypothesized to be involved in an intricate proteolytic cascade, through which they exert their physiological functions. Certain pathological manifestations of hKs is due to the perturbed regulation of hK proteolytic cascade.**

PURPOSE

- *In-vitro* identification of potential components of the hK proteolytic cascade:
  - Screening of heptapeptide library, representing the cleavage motifs of the 15 hKs.
  - Devising activity assays to test activation of full-length recombinant pro-hKs by active recombinant hK5.

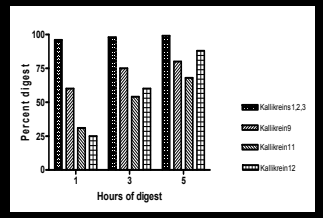
METHODOLOGY



- 1*- When the downstream kallikrein has a known specific substrate.
- 2*- When the coating antibody recognizes the mature form and does not block its activation motif.
- 3*- When the ABP recognizes the activated kallikrein and there is a size difference between active hK5 and the kallikrein of interest.

RESULTS

A



B

hK	1 hr [% digest]	3 hr [% digest]	5 hr [% digest]
1,2,3	96%	97%	98%
5	-	42	46
7	10	43	-
8	42	51	-
9	60	75	80
11	31	54	68
12	25	60	88

C

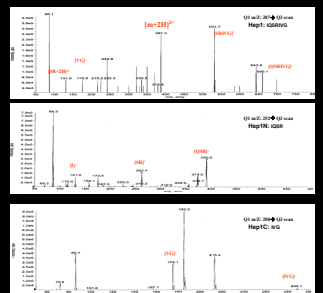
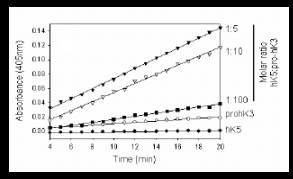


Figure 2: Cleavage of heptapeptides by active hK5. Heptapeptides representing the activation motifs of hK1-15 were digested with hK5 in a molar ratio of 1:1500 A,B). Relative cleavage efficiency of digests. Cleavage efficiency was measured as the percent area/height reduction of full-length Heps. C). Predicted cleavage sites were confirmed by MS.

RESULTS

A



B

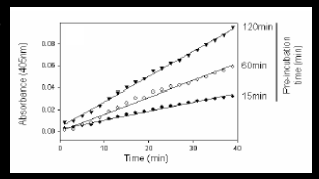


Figure 3: Activation of pro-hK3 by active hK5. A). hK5 was incubated (37°C) with pro-hK3 at molar ratios of 1:5, 1:10, and 1:100. B). hK5 was incubated (37°C) with pro-hK3 at the molar ratio of 1:1000 for 15, 60, and 120 minutes. Reactions were terminated by aprotinin at a molar ratio of 1:100. The activity of hK3 was measured, using the RPY-pNA substrate.

CONCLUSION

- Heptapeptide studies suggest that hK5 activates hKs 1,2, and 3 with a very high relative efficiency.
- hK5 Activation of pro-hK3 and pro-hK2 were confirmed, using full length recombinant proteins.
- Other candidate hKs activated by hK5 are hK 7,8, 9, 11 and 12.
- hK5 is possibly involved in a bidirectional regulation by activating and degrading excess active hK2 and hK3 .

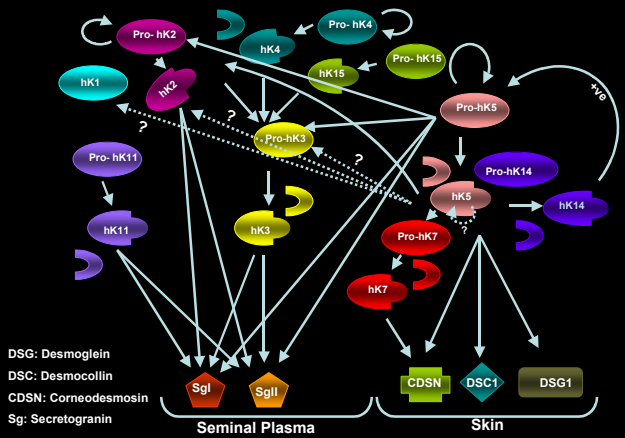


Figure 4: Schematic presentation of proteolytic cascade of human tissue kallikreins. Dotted arrows represent candidate components of cascade identified in this study.

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