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Presentation Title:	**Characterization of human kallikrein 5 (hK5) substrate specificity using combinatorial peptide libraries**
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Human kallikrein 5 (hK5; encoded by *KLK5*) is a novel member of a family of secreted serine proteases known as human kallikreins. In the past we have shown that the gene (*KLK5*) is differentially expressed in breast and ovarian cancer. At the mRNA level, *KLK5* is a marker of unfavorable prognosis and at the protein level, serum hK5 is elevated in a proportion of breast and ovarian cancer patients. In addition to its association with cancer, hK5 is also implicated in skin physiology and pathobiology.

In order to understand its role during cancer progression and its physiology we aimed to examine its substrate specificity by using fluorogenic combinatorial peptide libraries. We used soluble positional protease substrate libraries, possessing amino acid diversity at the P4-P3-P2-P1 (P1-diverse library) and P4-P3-P2 (P1-fixed library) positions. Each substrate contained the fluorogenic leaving group 7-amino-4-carbamoylmethylcoumarin. By using the P1-diverse library we found that hK5 possesses trypsin-like activity, with P1 preference for Arg over Lys. We screened for hK5 preference in P2, P3 and P4 positions by using a P1-fixed library. Broad specificity was observed for P2 and P4 positions while for P3, hK5 had preference for the basic amino acids Arg and Lys.

We then performed bioinformatic analysis to identify potential physiological substrates containing preferentially cleavage motifs. The sequence [YFWGPV] - [RK] - [NSFAM] - R, corresponding to the P4-P3-P2-P1 positions respectively, was used to screen the non-redundant protein database of the National Center of Biotechnology Information using the program PATTINPROT (http://npsa-pbil.ibcp.fr). A number of potential extracellular protein substrates for hK5 were retrieved, including components of the extracellular matrix (ECM) (*e.g.* collagens, laminin), adhesion molecules (*e.g.* cadherins, integrins), proteases (*e.g.* metalloproteases), receptors, inhibitors, growth factors and hormones.

ECM and adhesion molecules are of particular interest since they are related to cancer progression, tissue remodeling and skin biology. Some adhesion molecules, such as desmocollins, have already been shown to be cleaved by hK5. Furthermore, we verified that hK5 was able to cleave *in vitro* ECM components, such as fibrinogen, fibronectin and laminin. Other potential substrates, such as proteases, growth factors and receptors, suggest that hK5 may have diverse roles and significant cross-talk with other physiological pathways.

In conclusion we characterized the substrate specificity of hK5 with combinatorial peptide libraries and verified that it is a trypsin-like protease, able to digest ECM and adhesion molecules. Our results suggest that hK5 might have a role in tissue remodeling and cancer progression.

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