**96th Annual Meeting**
**April 16-20, 2005**
**Anaheim/Orange County, CA**

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| **Abstract Number:** | 2975 |
| **Presentation Title:** | **Enzymatic profiling of human kallikrein 14, a candidate cancer biomarker, by phage display substrate and positional scanning peptide libraries** |
| **Presentation Start/End Time:** | Monday, Apr 18, 2005, 1:00 PM - 5:00 PM |
| **Board Number:** | Board #21 |
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Human kallikrein 14 (hK14; encoded by the *KLK14* gene) is a secreted serine protease, differentially expressed in breast, ovarian, prostate and testicular cancer. Several reports suggest that hK14 may have clinical utility as a cancer biomarker. However, its biological role is currently unknown. Thus, the aim of this study was to determine the substrate specificity and candidate physiological targets of hK14, as a first step towards delineating its function. Recombinant hK14 was expressed in *P. pastoris* as the mature enzyme and purified to homogeneity from the culture supernatant by cation exchange chromatography. The substrate specificity of hK14 was then defined by screening: 1) a phage-displayed random pentapeptide library with exhaustive diversity and 2) combinatorial fluorogenic synthetic tetrapeptide positional scanning libraries with amino acid diversity at P1-P4 positions. Six rounds of phage-displayed library screening yielded 32 candidate pentapeptide substrates for hK14. Twenty-two of the selected peptides had Arg or Lys residues at the P1 position while the remaining 10 had Tyr, suggesting that hK14 has both trypsin and chymotrypsin-like specificity. The SwissProt database was screened with the most efficiently cleaved pentapeptides and several putative protein substrates for hK14 were identified, including matrilin-4, laminin and collagen IV. Full-length laminin and collagen IV proteins were shown to be efficiently fragmented by hK14 *in vitro*. The N-terminal P1-P4 specificity of hK14 was further assessed with combinatorial fluorogenic tetrapeptide libraries. hK14 demonstrated trypsin-like specificity with a P1 preference for Arg over Lys, in accordance with the phage display data. However, chymotrypsin-like specificity was not observed. Furthermore, hK14 exhibited broad specificity at P2 and P3, with a preference for aromatic amino acids (Tyr, Trp and Phe) at P4. These results were confirmed using several synthetic fluorogenic tetrapeptides. Two substrate cleavage motifs were designed based on the P1-P4 residues most preferred by hK14 and used to screen the non-redundant protein databases of the National Center for Biotechnology Information for potential hK14 targets, using the “Pattinprot” algorithm (http://npsa-pbil.ibcp.fr). Many putative substrates for hK14 were retrieved including adhesion molecules (cadherins, integrins), structural components of the extracellular matrix (collagens, laminins) and proteases [matrix metalloproteases (MMPs), transmembrane serine proteases (TMPRSSs)]. Collectively, our findings suggest that hK14 may preferentially act upon extracellular matrix proteins and might therefore be implicated in tissue remodeling and/or tumour progression, acting alone or synergistically with other proteases. Further studies are required to verify these results and the role of hK14 *in vivo*.

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