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**Presentation Title:** Regulation of the proteolytic activity of human kallikrein 5 (hK5) by tissue and plasma inhibitors

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Human kallikrein 5 (hK5; encoded by the *KLK5* gene) is a member of the human kallikrein (hK) family, which consists of 15 secreted serine proteases. hK5 is differentially expressed in breast and ovarian cancers. Recent studies have shown that *KLK5* is a marker of unfavorable prognosis and that serum hK5 levels are elevated in a proportion of breast and ovarian cancer patients. We have previously shown that hK5 is able to cleave extracellular matrix (ECM) components indicating that it may play role in cancer progression and metastasis. Furthermore, hK5 plays a role in skin desquamation and skin diseases by cleaving intercellular adhesion molecules, such as desmoglein, desmocollin and corneodesmosin.

The aim of this study is to examine the regulation of hK5 by plasma and tissue inhibitors. We examined the effect of a variety inhibitors including the serine proteinase inhibitors (serpins)  $\alpha_2$ -antiplasmin, antithrombin,  $\alpha_1$ -antitrypsin,  $\alpha_1$ -antichymotrypsin and the high molecular weight inhibitor  $\alpha_2$ -macroglobulin. We found that hK5 is inhibited by  $\alpha_2$ -antiplasmin and antithrombin, through an irreversible competitive-type mechanism, with a second-order rate constant,  $k_{+2}/K_i$ , of  $1 \times 10^{-2} \text{ M}^{-1} \text{ min}^{-1}$  and  $4 \times 10^{-4} \text{ M}^{-1} \text{ min}^{-1}$ , respectively.  $\alpha_2$ -macroglobulin was also able to inhibit 50% of hK5 activity at a 1:100 molar ratio.

*Serine protease inhibitor Kazal-type 5 (SPINK5)*, the gene product is also known as lympho-epithelial Kazal-type-related inhibitor; LEKTI) is a high molecular weight protein containing 15 potential inhibitory domains, including two classical Kazal-type domains and 13 non Kazal-type domains. SPINK5 protein has been linked to Netherton syndrome, a congenital skin disease in which mutations in *SPINK5* gene lead to truncated SPINK5 protein forms, containing fewer inhibitory domains. This results in higher protease activity in the stratum corneum and ultimately, to over-desquamation. By *in situ* hybridization we have previously shown that *KLK5* and *SPINK5*, at the mRNA level, colocalize in the stratum granulosum of normal epidermis and appendages. Here, we examined the inhibitory effect of recombinant SPINK5 domains 6 to 9 (sp6) and 9 to 12 (sp9). Both domains effectively inhibited hK5, sp6 through a noncompetitive-type mechanism and sp9 through a competitive-type mechanism, with inhibitory constants ( $K_i$ ) of  $5.32 \pm 0.27$  and  $1.62 \pm 0.12$  nM, respectively. Thus, SPINK5 is likely the major physiological inhibitor of hK5 in skin and possibly other tissues. We conclude that the serpins  $\alpha_2$ -antiplasmin and antithrombin and the high molecular inhibitor SPINK5 are likely the main regulators of hK5 activity in plasma and tissues, respectively. Disregulation of this balance may lead to cancer progression through the ability of hK5 to cleave ECM components, and to skin diseases through the ability of hK5 to cleave adhesion molecules.

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