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Kallikrein 14 Signalling through Proteinase-Activated Receptors

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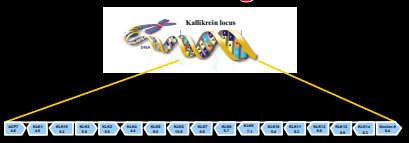
OVERVIEW

- **Proteinase-activated receptors (PARs):** a family of G-protein coupled receptors activated by serine proteinases via a proteolytically revealed 'tethered ligand' (Fig. 1). Four family members (Fig. 2); PARs 1, 2 and 4 signal to cells.
- **Human kallikreins (hKs):** A 15-member family of secreted serine proteinases implicated in tumour progression and cell survival (Fig. 4).
- **hK14:** *a tryptic kallikrein;* wide tissue distribution, implicated in breast and ovarian cancer (Fig. 5 and 6).
- **The mechanism of kallikrein action is not yet known:** Although some targets have been identified (e.g. extracellular matrix; pro-UPA), the mechanism whereby kallikreins regulate tissue function is not known.
- **We hypothesized that hK14, as a prototype kallikrein, modulates cell survival and tumour growth by regulating (activating or inactivating/dis-arming) proteinase-activated receptor (PAR) signalling.**
- **Main conclusions:** hK14 activated PAR_2 in cultured cells (Ca^{++} signalling) and caused PAR_2-mediated relaxation of rat and murine vascular tissue. In addition, hK14 had a dual action on PAR_1, depending on the enzyme concentration (principally dis-arming). In human platelets, hK14 was able to cause aggregation by activating PAR_4 whilst dis-arming PAR_1.
- **Significance of study:** In the setting of human tumours, known to be platelet-rich, hK14 would trigger platelet aggregation and the preferential release of platelet endostatin rather than VEGF via PAR_4 (PNAS 2005;102(1):216-20). Also, by targeting PAR_2- and/or PAR_4, hK14 could participate in tumour growth and survival, as well as in the inflammatory responses during cancer progression.

Figure 4: Human Kallikreins



Characteristics of the kallikrein family:

- The largest cluster of continuous proteinases of any kind
- The largest group of serine proteinases
- Trypsin or Chymotrypsin-like activity

Borgono CA. et al, Nat Rev Cancer. 2004;4:876-90
Borgono CA. et al, Mol Cancer Res. 2004;2:257-80

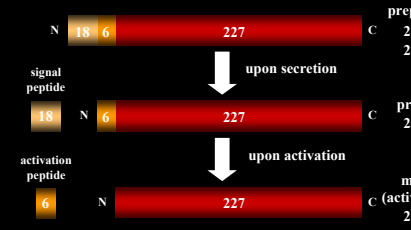
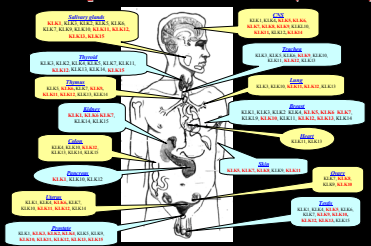


Figure 5: Kallikreins are secreted enzymes e.g. hK14

Figure 6: Kallikrein expression

Tissue Expression of Kallikreins (RT-PCR)



Kallikreins and cancer

- PSA/hK3 is utilized to monitor prostate cancer patients
- hK6 (Zyme / proteinase M / Neurosin), hK10 (NES1), hK11, hK8, hK5 and hK14 may represent novel ovarian cancer biomarkers
- hK11 may represent a novel prostate cancer biomarker
- hK5 and hK14 may represent novel breast cancer biomarkers
- hKs can cleave several pro-uPA, GFs and ECM proteins

Figure 1: Mechanism of PAR activation (activation by proteolysis)

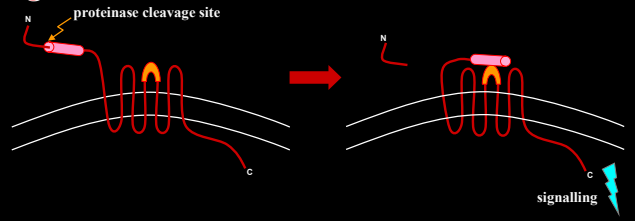


Figure 2: The PAR family

4 PARs are known in rodents and humans

Receptor	Major Activating proteinases	Some Disarming proteinases
PAR_1	Thrombin	Trypsin, Cathepsin G
PAR_2	Trypsin, Tryptase	Elastase
PAR_3	Thrombin	Cathepsin G
PAR_4	Thrombin	Unknown; Kallikreins ?

Steinhoff M. et al., Endocr Rev. 2005;26:1-43

Figure 3: Proteinase cleavage/activation site

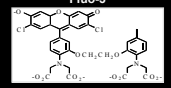
Note: Hirudin-like binding domain unique to PAR_1 (and PAR_3)

Materials and Methods

Calcium signalling

- Calcium signalling assay in human HEK cells (PARs 1 and 2 / PAR_4) and rat KNRK (PAR_2)
- Method: Incubate cells with Ca^{2+} indicator (Fluo-3) → examine for cross-desensitization of PARs, using PAR-activating peptides, agonists and antagonists:
  - $TFLLR\text{-}NH_2$ - selective desensitization of PAR_1
  - $SFLLR\text{-}NH_2$ - selective desensitization of PARs 1, 2
  - $AYPGKF\text{-}NH_2$ - selective desensitization of PAR_4
  - Trypsin and / or Thrombin
  - Phenylephrine and Acetylcholine
  - L-NAME - inhibitor of NOS-mediated aorta relaxation

Fluo-3



Bioassays

- Aorta endothelium: Contraction / relaxation (PAR_2)
- Human (PAR_1 and PAR_4) and Rat (PAR_4) platelets: Aggregation / Ca^{++} assays
- Inflammation

Kawabata A. et al., JPET 1999;288: 358-70
Hollenberg MD. et al., Can J Physiol Pharmacol. 1997;75:832-41
Hollenberg MD. et al., Can J Physiol Pharmacol. 2001;79:439-42
Vergnolle N. et al., Br J Pharmacol. 1999;127:1083-90

Results

1. hK14 can cleave within synthetic PAR 1, 2 and 4 peptides (designed based on the cleavage/activation motifs; Fig.3)

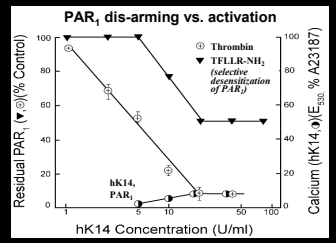
Tethered ligand sequence

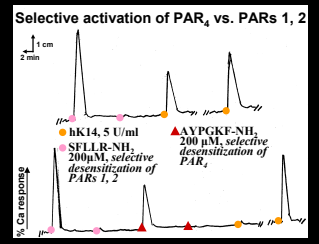
Receptor	Trypsin (3.5 U/ml)	hK14 (4.3 U/ml)
$hPAR_1$	NATLDPR / **SFLLRN**PNDKYE	NATLDPR / **SF / LLR** / NPNDKYE
$hPAR_2$	GTNRSSKGR / **SLIGK / V / DGTSH VTGK / GVT**	GTNRSSKGR / **SLIGK / VDGTSHVTGK / GVT**
$hPAR_4$	GDDSTPSILPAPR / **GYPGQV**	G / DDSTPSILPAPR / **GY / PGQV**
$rPAR_2$	GPNSKGR / **SLIGRLDTPY** / G / GC	GPNSKGR / **SLIGRLDTPY** / GG / C
$rPAR_4$	L / NESK / SPDKPNPR / **GFPGKP**	LNESKSPDKPNPR / **GFPGK / P**

Only the *intact ligand sequences*, that would remain tethered to the receptor, would result in signalling. Other potential cleavage sites would dis-arm the receptor, preventing receptor activation.

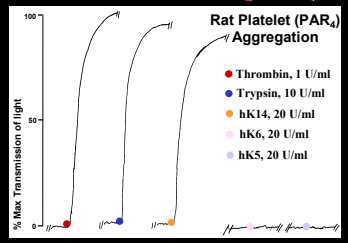
Oikonomopoulou K. et al., Biol Chem 2006

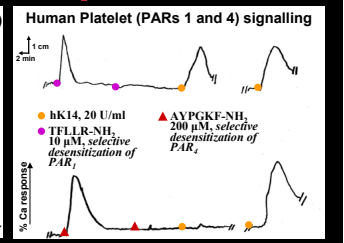
2. Kallikrein 14 can selectively disarm PAR_1 (lower concentrations), whilst activating PARs 2 and 4; Ca^{2+} in HEK cells



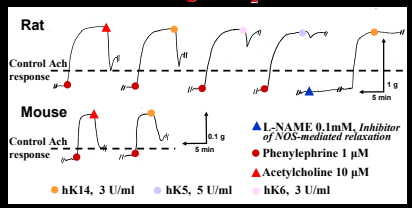


3. Kallikrein 14 can cause platelet aggregation and Ca^{2+} signalling through PAR_4 in isolated platelets

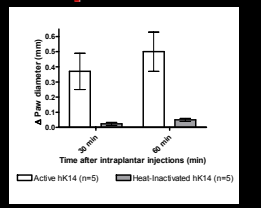




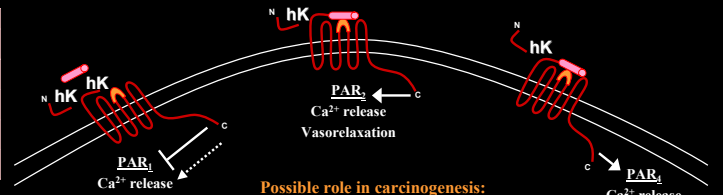
4. Kallikreins can cause aorta relaxation, mediated through PAR_2 activation



5. hK14 can cause murine paw inflammation



Conclusions; Kallikreins can regulate PAR activity



Possible role in carcinogenesis:

- Angiogenesis
- Tumour growth
- Inflammation
- Tumour survival