Tumour-derived proteinases and signaling: Kallikrein-mediated PAR activation and inflammation

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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 and 3); PARs 1, 2 and 4 signal to cells.
- Human kallikreins (KLKs): A 15-member family of secreted serine proteinases implicated in tumour progression and cell survival (Fig. 4).
- For example, KLK14: *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 kallikreins, considering KLK14 as a prototype kallikrein, modulates cell function by regulating (activating or inactivating/dis-arming) proteinase-activated receptor (PAR) signalling.
- Main conclusions: Kallikreins activated PAR_2 in cultured cells (Ca^{++} signalling) and caused PAR_2-mediated relaxation of rodent vascular tissue. In addition, KLK14 had a dual action on PAR_1, depending on the enzyme concentration (principally dis-arming). In human platelets, KLK14 only was able to cause aggregation by activating PAR_4 whilst dis-arming PAR_1. When administered *in vivo*, KLK14 caused a paw oedema response comparable in magnitude and time course to that generated by trypsin.
- Significance of study: Our data demonstrate that by activating PARs 2 and 4 and by inactivating PAR_1, tumour-derived kallikreins, like KLKs 5, 6 and 14, may play a role in regulating cancer cell signaling and tumour-induced inflammatory response.

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

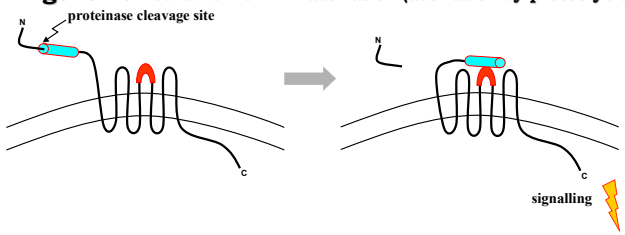
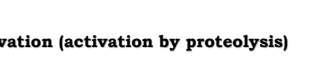


Figure 2: The PAR family

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 ?

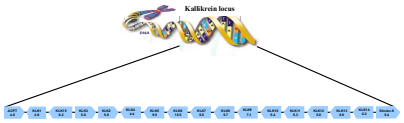
Steinhoff M. et al., Endocr Rev. 2005;26:1-43

Figure 3: Proteinase cleavage/activation site



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

Figure 4: Human Kallikreins

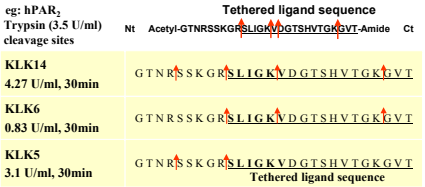


Within the human genome kallikreins represent:
- The largest cluster of continuous serine proteinases of any kind
- The largest group of serine proteinases
- Trypsin or Chymotrypsin-like activity

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

Results

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



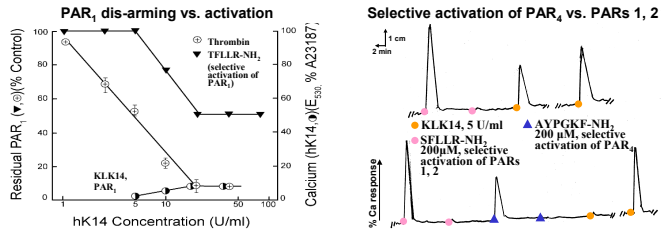
Conclusion:

Kallikreins can cleave within the N-terminal sequences of PARs 1, 2 and 4 at sites that could lead *either to receptor activation OR to disarming of the receptors*

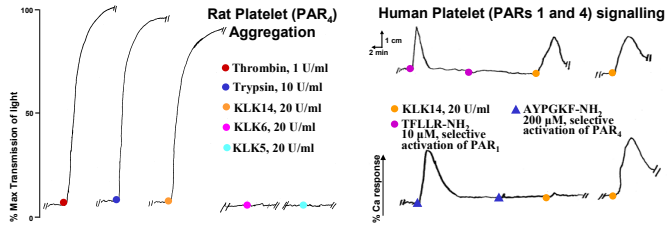
Only the tethered ***underlined sequences*** would result in signalling. Other potential cleavage sites would dis-arm the receptor.

Oikonomopoulou K. et al., Biol Chem 2006;387:817-24
Oikonomopoulou K. et al., JBC 2006;281:32095-112

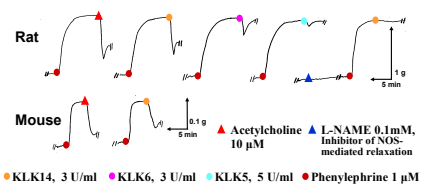
2. Kallikrein 14 can selectively disarm PAR_1, and activate PARs 2 and 4



3. Kallikrein 14 can cause platelet aggregation and signalling (PAR_4)



4. Kallikreins can cause aorta relaxation, through PAR_2 (confirmed with PAR_2 -/- mice)



5. Kallikrein 14 can cause mouse paw inflammation

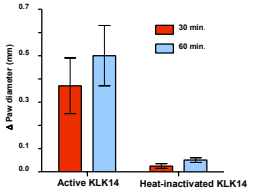
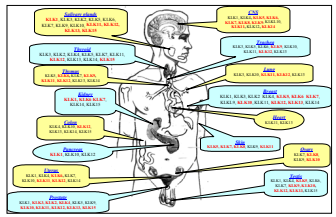


Figure 5: Kallikreins are secreted enzymes e.g. KLK14

Figure 6: Kallikrein expression

Tissue Expression of Kallikreins



Kallikreins and cancer

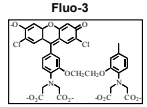
- PSA/KLK3 is utilized to monitor prostate cancer patients
- KLK6, KLK10, KLK11, KLK8, KLK5 and KLK14 may represent novel ovarian cancer biomarkers
- KLK11 may represent a novel prostate cancer biomarker
- KLK5 and KLK14 may represent novel breast cancer biomarkers
- KLKs can cleave pro-uPA, GFs and several ECM proteins

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 and agonists

Fluo-3



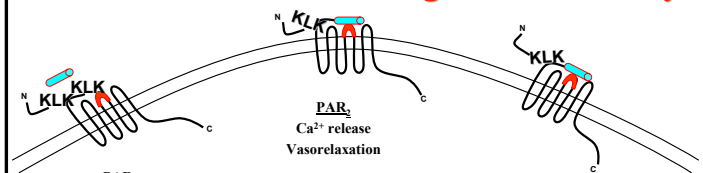
Kawabata A. et al., JPET 1999;288: 358-70

Bioassays

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

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

Conclusion: Kallikreins can regulate PAR activity



Possible role in carcinogenesis:

- Angiogenesis
- Tumour growth
- Inflammation
- Tumour survival