Targeting of prostate cancer with peptides to prostatic proteases

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Kallikrein-related peptidases (KLK)

- In human 15 highly conserved serine proteases
  - Clustered on 19q13.3–13.4
  - PSA and hK2 only in primates (not in mouse or rat)
- Single-chain secreted preproenzymes (~30–40 kDa)
- Trypsin-/chymotrypsin-like specificities
- KLK genes are often coexpressed
  - Proteolytic cascades?
- Potential cancer biomarkers
  - Prostate cancer: KLK2–6, 10, 11, and 13–15
- Drug & imaging targets
  - PSA & hK2 activable prodrugs
  - PSA-inhibitors (also for imaging?)
  - Other kallikrein inhibitors (modified serpins etc)
Prostate-specific antigen (PSA, KLK3)

- Serine protease with restricted chymotrypsin-like activity (preferentially cleaves after Tyr residue)
  - Member of kallikrein family
- High expression in prostate
- Prostate cancer marker
  - Expression decreased in prostate cancer
  - High serum levels due to increased release into circulation
  - Not tumor specific
- Degradation of seminal clot
PSA in circulation

- Majority complexed with protease inhibitors
- Some as free enzyme, but mostly inactive
- Very low level of active PSA in circulation
Does PSA affect prostate cancer growth?

- May promote tumor growth by
  - Enhancing cell proliferation and invasion
    - "Activation" of growth factors (IGF-1, TGF-β)
    - Degradation of ECM components

- May prevent tumor growth by
  - Inhibiting angiogenesis both in *in vitro* and *in vivo* models
  - Inhibiting the formation of metastases

- Clinical observations
  - PSA expression decreases with increasing tumor grade and aggressiveness
  - Low tissue concentrations of PSA associated with poor prognosis
  - High PSA expression related to low microvessel density

- Can we utilize the biological activity of PSA clinically?
  - Would enhanced activity slow down prostate cancer growth?
  - Could explain slow growth of prostate cancer
Model for development of prostate cancer

- **Age (years)**
  - 0,01
  - 0,1
  - 1
  - 10
  - 100
  - 1000

- **Tumour size (mm)**
  - 0,01
  - 0,1
  - 1
  - 10
  - 100
  - 1000

- **Tumor diameter**
- **Need for vascularization**
- **Slow growth, DT 2 years**
- **Rapid growth**
- **Lethal stage**
- **Symptoms and clinical diagnosis**
- **Detection by screening**
- **Latent stage**
- **Prostatic intraepithelial neoplasia**
Benzoxacinone derivatives

Triazole containing family
Selection of peptides by phage display

Library construction

- Screen phage library with PSA
- Remove nonspecific phage by washing
- Elute specifically bound phage
- Amplify eluted phage

- Determine sequence of selected phage

Legend

- Phage
- Phage DNA
- PSA-binding peptide
- Non PSA-binding peptides
- Antibody to PSA
- Free PSA

>10^9 clones
### PSA binding peptides

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<tr>
<th>Code</th>
<th>Origination library</th>
<th>Peptide sequence</th>
<th>No. of isolates</th>
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</tbody>
</table>

50% stimulation of PSA activity (µM):
- 2.2
- 1.7
- 0.57
Immunopeptidometric assays for active PSA

**GST-fusion peptide, Fluorometry** (Wu et al., Clin Chem 2004)

**Proximity ligation** (Zhu et al., Biol Chem 2006)
With protein substrates B2 stimulates the activity of KLK3 more effectively than C4.

With peptide substrates C4 stimulates the activity of KLK3 more effectively than B2.
Binding specificity of the peptides

Interaction with active PSA and proPSA

Interaction with proteinases

Interaction with PSA-serpin complexes
PSA stimulating C4-peptide enhances antiangiogenic effect of PSA

HtVEC: PSA 0.1 µM, C4-peptide 100 µM. Matrigel plug: PSA 1 µM, C4-peptide 50 µM
IHC staining of LNCaP xenograft tumors

- PSA x100
- Ki-67 x100
- CD31 x400
- AR x200
Design of the B-2 treatment experiment

- Injection every 2nd day
- 0.2 mg B-2 peptide subcutaneously
- Serum collection once per week
After 5 weeks of treatment

LNCaP bearing NUDE Buffer group

LNCaP bearing NUDE B-2 peptide group
**LNCaP tumors** (high PSA)

- **B2-peptide**
- **buffer**

**22RV1 tumors** (low PSA)

- **B2-peptide**
- **buffer**

**aP tumors**

- **Ctrl pep.** (n=18)
- **C4** (n=19)
Peptide drugs

- Ca. 700 peptide drugs under development
- Many hormones, messengers and biomediator molecules are amino-acid based

Challenges
- Expensive to produce
- ADME

Advantages
- Specificity/selectivity
- Targeting of new mechanisms
- Target validation
Peptide ADME

- **Adsorption**
  - poor oral pharmakokinetics (i.e., i.v. administraton)
  - poor permeation

- **Distribution**
  - should be OK for our purposes (preferably no BBB penetration)

- **Metabolism**
  - broken down in the gut
  - sensitive to proteolysis
  - generally no toxic metabolites

- **Excretion**
  - fast (renal filtration)
  - accumulation in non-targeted organs & tissues

- Properties can be modified
Ways to improve properties

- Pegylation (or other polymers)
  - increased size (>40kDa) decreases renal filtration
  - decreased proteolysis and immunogenicity
- Backbone cyclization
  - stability
  - sometimes oral bioavailability
- D-amino acids, peptidomimetics
  - stability
Development of better compounds

- Peptides are not optimal as drug molecules
- We aim to develop more drug like molecules that are suitable for *in vivo* use
  - HTS, ca. 50,000 cpds, 2 inhibitors found
  - Structure/binding site determination
  - Molecular modeling (Henna Härkönen, Antti Poso)
  - Structure-based drug design
  - Virtual screening, novel PSA-activity stimulating compounds
  - Peptidomimetics (Erik Wallén, Kristina Luthman)
    - Use privileged structures as scaffolds and other methods of lead optimization adapted from medicinal chemistry
Peptid structure required for binding to PSA

Single Ala replacements
Multiple Ala replacement of C-4

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Activity</th>
<th>Purity (%)</th>
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PSA stimulating peptide B-2

Bridge structure replaced with γ-amino butyric acid and aspartic acid

Improved stability without losing bioactivity
Conclusions

- Specific peptides stimulating PSA-activity can be made
  - Purification of enzymatically active PSA
  - Development of a novel quantitative assays
  - Not stable & excreted quickly
- The peptides stimulate antiangiogenic effect of PSA
- Stimulation of enzymatic activity of PSA might slow down prostate cancer growth
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