ERK5 and osteosarcoma

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ERK5 is a signalling protein

MEK5/ERK5 pathway: The first fifteen years Barbara A. Drew, Matthew E. Burow and Barbara S. Beckman
ERK5 and osteosarcoma

Pre-clinical evidence
Silencing ERK5 in osteosarcoma cell lines affects:
- invasiveness in vitro
- Slug/MMP9 expression in vitro

Clinical evidence
Overexpression of ERK5 (MAPK7) associated with
- poorer response to treatment (chemotherapy)
- tumour progression (metastases)
- worse overall survival

ERK5 and osteosarcoma

1. How does ERK5 regulate osteosarcoma?
2. Can we target ERK5 for therapeutic gain in osteosarcoma?

Preclinical model of osteosarcoma
IF injection of 143-B cells, metastatic to the lung

Experimental design

ANALYSIS
- Imaging signal
- FACS
- qRT-PCR
- ELISA
- Lung clonogenicity
- Histopathology
Loss of ERK5 impairs osteosarcoma growth

- **Tumour burden**
  - Control
  - shERK5

- **MMP9 activity**
  - Control
  - shERK5

- **PET imaging** $^{18}$F-DPA-714
  - Control
  - shERK5

- **Macrophages**
  - Control
  - shERK5

- **Microscopy**
  - Tumour
  - Macrophages MMP-9
ERK5 is required for the metastatic spread of osteosarcoma

<table>
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<tr>
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<th>Number of colonies/mg of lung</th>
<th>SD</th>
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<tr>
<td>WT-luciferase</td>
<td>4.43 ± 0.46</td>
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<tr>
<td>shERK5-luciferase</td>
<td>0.092 ± 0.082</td>
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**Ex-vivo imaging, excised lung, control mice**

- Tumour burden
  - BLI imaging
  - MMP9 activity
  - FLI imaging

No signal detected in shERK5 lungs
Underpinning mechanism

Loss of EKR5 in 143B cells
- Down-regulation of EMT genes
- Upregulation of E-cadherin
- Decreased pro-angiogenic signals
- Reduction in cytokine production

Corroborating in ex vivo samples
- IHC
- FACS analysis of immune cell composition
- Isolation tumour cells and macrophages (FACS)
  - ELISA
  - qRT-PCR

In vivo phenotype of EKR5 loss
- Prevention of metastatic spread
- Decrease in macrophage residency
- Slower primary tumour growth
Translation

- Small molecule degrader of ERK5
- Should target ALL the functions of ERK5
- Mimics the phenotype of ERK5 knockdown
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