# Multiplex kinase activity profiling to study mechanisms of bone metastasis in prostate cancer cells

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## Bone metastasis in prostate cancer

- In the UK, prostate cancer (PCa) will affect one in eight men and cause one death every 45 minutes[1]. Patients with PCa frequently develop bone metastases, associated with debilitating skeletal symptoms, decreased quality of life and reduced survival. Over 90% of men who die of PCa have bone metastases[2]
- Metastatic PCa cells home to the endosteal niche – the interface between the bone matrix and bone marrow – where they compete with haematopoietic stem cells (HSCs) for niche occupancy[3]. Osteoblasts are one of the primary cell types in this niche and it has been shown that PCa cells localise to osteoblast-rich areas[4], suggesting that osteoblasts are a key driver of bone metastasis
- Tumour cells are thought to exploit similar mechanisms to HSCs to reach this niche and survive within it, such as CXCL12/CXCR4 and Annexin II signalling[3,5]. However, our understanding of the mechanisms of crosstalk between osteoblasts and PCa cells is incomplete
- We hypothesise that by studying changes in PCa cell signalling in response to osteoblast-secreted factors, we can identify pathways to target to disrupt bone metastasis in patients

## hFOB1.19 cells as a model of human osteoblasts

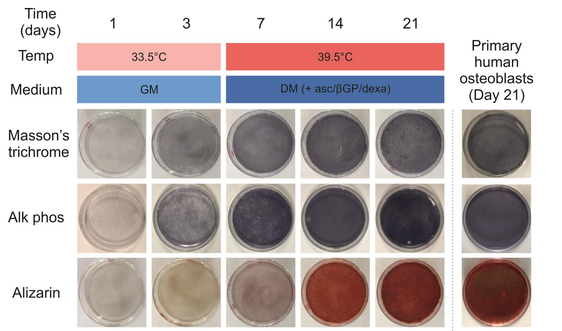


**Figure 1. hFOB1.19 are a suitable model of human osteoblasts.** As with primary human osteoblasts, treatment of post-confluent hFOB1.19 cells with ascorbate, β-glycerolphosphate and dexamethasone promotes a mature osteoblast phenotype, characterised by collagen deposition, high alkaline phosphatase activity and an ability to mineralise their extracellular matrix. hFOB1.19 cells have been conditionally immortalised with a temperature-sensitive SV40 vector and proliferate rapidly at 33.5$^0$C and stop growing at 39.5$^0$C.

## Cytokine- and chemokine-rich conditioned medium (CM) from mature osteoblasts promotes invasion in prostate cancer cells

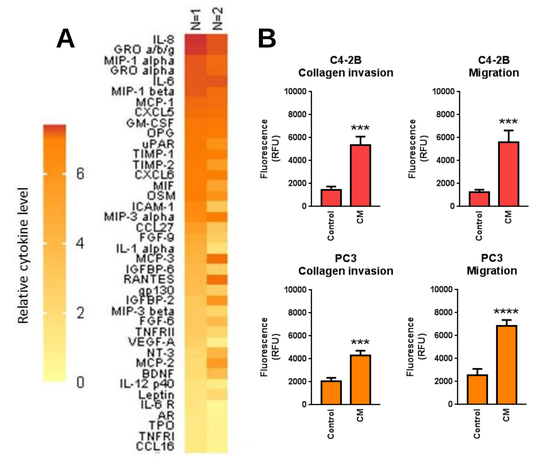


**Figure 2A. Serum-free CM collected from hFOB1.19 cells at day 21 is rich in cytokines and chemokines,** including typical osteoblast-derived factors such as IL-8, IL-6 and OPG which regulate the bone remodelling cycle in normal physiology. The top 40 most abundant cytokines out of an antibody array of 120 human cytokines are shown. **2B. Osteoblast CM promotes chemotactic cell migration and invasion in PCa cell lines**, as measured by transwell invasion assay. Osteoblast CM did not induce chemokinetic motility (data not shown). Statistical significance ($p < 0.05$) was determined by t-test.

## Profiling kinase activity using the PamGene platform

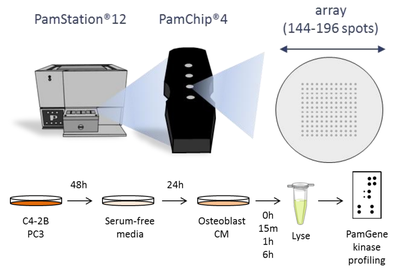


**Figure 3. Outline of PamGene technology and our experimental workflow.** PamGene arrays utilise a 3D aluminium oxide membrane spotted with peptides containing literature derived or computationally predicted phosphosites for Tyr or Ser/Thr kinases. Fluorescent phospho-specific antibodies are used to detect activity of kinases present in the sample.

## PamGene technology identifies putative invasion-related kinases and pathways

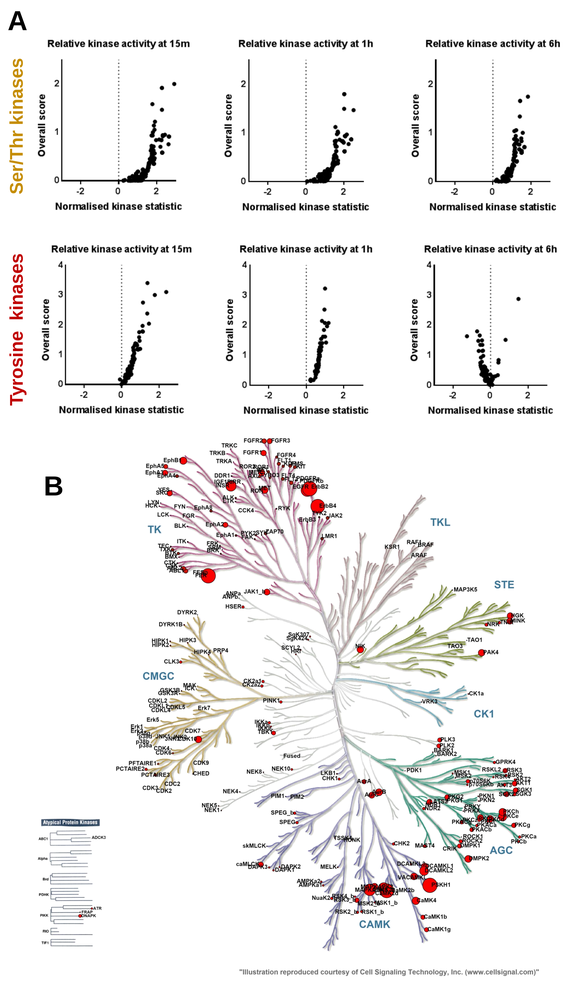


**Figure 4A. Osteoblast-secreted factors modulate kinase activity in PC3 prostate cancer cells.** Normalised kinase statistic represents the relative change in activity of each kinase; overall score provides a combined measure of significance and specificity (where the higher the specificity the less likely it is that the same kinase statistic could be observed with a random set of peptides from the array). **4B. 15m results for PC3 mapped onto the kinome.** Some up-regulated kinases – e.g. Eph RTKs, RSKs, ROCKs, PAK4 and FER – have previously been shown to play a role in chemotaxis or invasion in PCa cells or other cancer types; others – such as PSKH1 – have not been explored in the context of metastasis.

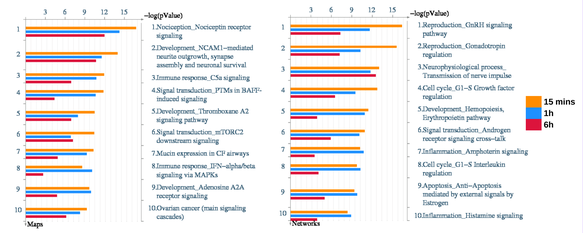


**Figure 5. GeneGo pathway analysis identifies pathways (left) and networks (right) enriched in osteoblast CM-stimulated PC3 cells,** based on significantly altered peptides ($p < 0.05$; ANOVA) at each time-point. Enrichment for nociception and neuronal biology pathways likely reflects the increased activity of $Ca^{2+}$/calmodulin-dependent kinases (CaMKs) observed; these kinases have also previously been shown to regulate cell migration and invasion[6,7].

## Conclusions

- PamGene kinase activity profiling provides a useful discovery platform to identify convergent kinases, phosphoproteins or pathways activated or suppressed in response to complex stimuli (i.e. osteoblast CM)
- Using this approach, we have identified a number of putative invasion-related kinases for further validation
- Ultimately, inhibition of pathways driving endosteal homing may delay the formation of new bone metastases in PCa patients or drive tumour cells out of the protective osteoblastic niche, potentially sensitising them to cytotoxic chemotherapy

## Ongoing work

- Functional in vitro and in vivo validation of kinases of interest by siRNA knockdown/CRISPR deletion, over-expression and pharmacological inhibition
- ITRAQ mass spectrometry-based phosphoproteomics timecourse of PCa cells treated with osteoblast CM

## References

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