49 EphA2 ligand independent activation underpins PTEN related metastatic migration and poor outcome in prostate cancer

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Brown M. [1] , Hart C. [1] , Ali A. [1] , Sachdeva A. [1] , Clarke N. [2]

[1]University of Manchester, Genito Urinary Cancer Research Group, Manchester, United Kingdom, [2]The Christie, Dept. of Surgery, Manchester, United Kingdom

Introduction & Objectives: The molecular mechanisms supporting prostate cancer (PCa) metastasis are poorly understood. We have shown previously that the poly-unsaturated fatty acid arachidonic acid (AA) induces the key mesenchymal to amoeboid transition in PCa through ligand independent activation of EphA2, enabling transendothelial migration and early invasion of bone marrow. This study further investigates EphA2 signalling in relation to PTEN, migrational biopotential and clinical outcome in PCa.

Materials & Methods: PC-3 EphA2 ligand dependent and independent activity was knocked out by CRISPR generating EphA2$^{\Delta Asp739}$ and EphA2$^{\Delta Ser897}$ cell lines respectively. PTEN positive PC-3 cells and siRNA PTEN knockdown DU145 cells were studied using standardised invasion and transendothelial migration assays. The EphA2 signalling cascade was interrogated using Western blotting. Results were matched using TMA-based primary biopsy tissue from 532 PCa patients discovery (n=280), validation (n=252) followed for up to >15 years. TMAs were stained for EphA2, pEphA2^{Ser897}, PTEN and pan cytokeratin and expression linked to clinicopathological features.

Results: Stimulation of PC-3 cells with AA increased pAkt within 5 minutes, with subsequent phosphorylation of EphA2^{Ser897} (ligand independent pathway) and transendothelial migration. Expression of PTEN significantly reduced pAkt within PC-3 cells, preventing EphA2^{Ser897} phosphorylation and inhibiting transendothelial migration. AA had no effect on ligand dependent EphA2 activation in either PTEN^{+ve} or PTEN^{-ve} PC-3 backgrounds. Knockdown of PTEN in DU145 cells increased invasion in a manner similar to that seen in PC-3 cells. In TMA analysis, EphA2 expression correlated with higher Gleason grade and a poorer cancer specific survival (136 vs 85 months, HR 0.5226 95% CI 0.3437 – 0.7948; p=0.0024). This correlation with poor survival was enhanced by combining pEphA2^{ser897} (ligand independent signalling) staining with loss of PTEN (36 vs 109 months, HR 2.20 95% CI 1.48 - 3.27; p < 0.0001).

Conclusions: The key process of mesenchymal to amoeboid transition, enabling PCa transendothelial migration and metastasis, is driven by omega-6 poly-unsaturated fatty acid induced phosphorylation of EphA2 by pAkt. This is enhanced with PTEN loss. Detection of ligand independent pEphA2^{ser987} signalling in a PTEN null background in primary PCa biopsies is strongly prognostic for poorer survival.