Purinergic signalling in the prostate cancer metastasis initiating cells

Project leader: Dr Ning Wang

Co-supervisor: Miss Freyja Docherty

Research proposal

Bone metastasis is a devastating clinical problem affecting 1.5 million cancer patients worldwide each year. Prostate cancer is currently the most commonly diagnosed cancer (34,000 cases annually) and the second leading cancer death in man (10,000 cases annually) in the UK. About 90% of patients with advanced disease have bone metastasis and this is the main cause morbidity in these patients, rather than the effects of the primary tumour. The latter can be treated surgically and with radiotherapy but approaches for treating bone metastasis are limited due to their disseminated nature. Preventing the occurrence of bone metastases is currently not possible due to poor understanding of the mechanism leading to tumour colonization of bone. In prostate cancer, it is suggested only a small percentage of tumour cells (<1%) within a primary cancer have the ability to leave the primary site, survive in the circulation, and even a smaller subset of these cells (~<0.02%, so called metastasis initiating cells) form metastases in bone. Acquiring an epithelial-mesenchymal transition (EMT)-like phenotype has been suggested as essential process required for these tumour cells to go through the multi-step metastasis process (1).

Purinergic receptors, the receptors of adenosine and adenosine triphosphate (ATP), have been shown to be key players in tumour microenvironment regulation, host-tumour interaction, and the modulation of immune cell responses and cytokine release (2). More interestingly, a recent study in a breast cancer model has suggested the gene expression alterations of P2 receptors (including seven P2X receptors: P2X1-X7, and eight P2Y receptors: P2Y1, Y2, Y4, Y6, Y11, Y12, Y13, Y14) was involved in EGF-induced EMT in MDA-MB-468 breast cancer cells (3). In the proposed study we will determine whether alteration of gene expression profile of P2 receptors is a feature of the metastasis-initiating phenotype in prostate cancer.

In our recent studies, a novel method has been developed to track metastasis initiating prostate cancer cells in bone metastasis xenograph models. Briefly, we have shown that metastasis-initiating cells are mitotically dormant and as a result can maintain high levels of the vital fluorescent lipophilic dyes (Vybrant DID) after single, in vitro staining. This allows the detection, isolation and characterisation of these cells in vitro and in vivo. We have confirmed their dormancy and EMT status using a combination of state-of-art techniques such as multiphoton microscopy, FACS sorting, Taqman qRT-PCR and immunohistochemistry. This has allowed us, for the first time, to partially characterize metastasis-initiating cells, relevant to the development of bone metastasis in patients where dormancy is followed by recurrence of growing metastasis. We do not however understand the factors that control dormancy or EMT status. In this study we aim is to further characterize metastasis initiating cells and focus on purinergic signalling as a regulator dormancy/EMT. In the SURE project we will compare the P2 receptors gene profile in metastasis initiating and non-metastasis initiating populations.
The proposed study will test the following hypothesis: The cell surface signature of P2 receptors is specific to metastasis initiating cells and EMT status in prostate cancer models.

To test the above hypothesis, three specific objectives need to be met:

**Objective 1. Basic lab training:** The successful candidate will be given training in basic research techniques and skills including literature review, prostate cancer cell line (PC3, LNCAP, and C4 2B4) culture in vitro, microscopy, and molecular biological techniques.

**Objective 2. Gene profile of P2 receptors in prostate cancer cells.** The candidate will perform RNA extraction from populations of prostate cancer cell lines including PC3, LNCAP, and C4 2B4, followed by cDNA synthesis and quantitative RT-PCR analysis using Taqman gene expression assays for the fifteen P2 receptors and EMT markers including N-Cadherin, Fibronectin and MMP3 etc. The gene expression profile of P2 receptor in prostate cancer cell lines will then be built after data analysed using SDS 2.2.1 software and ‘GenePattern’ web software.

**Objective 3. Gene profile of P2 receptors in metastasis initiating cells.** Vybrant DID labelled prostate cancer cells will be cultured for 14 days and the dormant population (~1%) will be isolated using the FACSaria cell sorting system. Total RNA will be then isolated using the Promega ReliaPrep RNA cell miniprep system and subject to cDNA synthesis and Taqman quantitative RT-PCR analysis. The gene expression profile will be compared with that of general population using statistical analysis software: GraphPad Prism 5.

The timeline of the research plan is showing in the following chart

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References:

