

Title: Developing tumour-activatable minicircles as novel reagents for prostate cancer detection

Trainee Name: TianDuo Wang

Supervisor(s): Dr. John Ronald

Structured Abstract:

Introduction: Early and accurate detection of prostate cancer (PCa) is critical for a positive patient outcome. Of particular importance to PCa is the ability to determine the aggressiveness of a tumour with minimal invasiveness, a limitation of current biomarker-based exams. In this work, we propose a novel non-viral gene construct called tumour-activatable minicircles (TA-MCs), which are shortened versions of plasmids that are less immunogenic and more potent. These TA-MCs are able to deliver a gene into cancer cells, forcing them, but not normal cells, to produce a unique and sensitive biomarker. We have designed TA-MCs that express secreted embryonic alkaline phosphatase (SEAP), a reporter protein detectable in blood. SEAP expression was mediated by the survivin promoter (pSurv), which is transcriptionally active only in cancers, ensuring our gene is only expressed in cancer cells. Together, these components only produce SEAP in the presence of cancer. Our objective was to build SEAP expressing TA-MCs and assess their ability to detect and characterize PCa across cell lines and in mice.

Materials and Methods: We first constructed parental plasmids, precursors to minicircles, that use pSurv to mediate expression of SEAP. Parental plasmids were then converted into TA-MCs using an established production system. We studied these TA-MCs in vitro via transfection of PCa cell lines (Du145, LNCaP, PC3, PC3MLN4) of varying aggressiveness, measuring SEAP in cell supernatant using commercially available kits. Next, we assessed TA-MCs in nude mice with subcutaneous PCa tumours (LNCaP, PC3MLN4). TA-MCs complexed with polyethylenimine was injected intratumourally, then SEAP levels were measured in blood samples and compared to tumour survivin levels. Survivin expression was measured in cell and tumour lysates using Western Blot.

Results: We found that PCa cells produced significantly more SEAP than normal prostate cells. Between PCa cells, SEAP secretion was positively related to cellular survivin expression, being highest in cells with the most survivin expression. Similarly, blood SEAP was significantly higher in mice with aggressiveness PC3MLN4 tumours than mice with non-aggressive LNCaP tumours.

Discussion: The results indicate that TA-MCs expressing SEAP can not only discern PCa from healthy prostate cells, but can also differentiate PCa cells with varying survivin expression. Notably, SEAP levels can successfully discriminate between tumours of different aggressiveness in mice. This system has potential to be a powerful method to distinguish patients with high-risk PCa from those with indolent prostate tumours. Our next step is to build and evaluate TA-MCs that express a gene called Gaussia luciferase, which is detectable in urine and may be more sensitive than SEAP. Furthermore, the modular nature of this technology leaves opportunities for implementation of other genes and so we hope to explore TA-MCs as tumour-specific therapeutic agents.