Title: Evaluation of tumour-activatable minicircles for prostate cancer diagnosis and therapy

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Structured Abstract:

Introduction: Gene-based technologies are an emerging approach for improved cancer diagnosis and treatment that is currently limited by tumour-specificity, delivery efficiency, and safety. To prevent off-target effects, groups have used tumour-specific promoters such as the survivin promoter (pSurv) to drive expression of downstream genes in cancer cells. In prostate cancer (PCa), importantly, pSurv activity has been correlated to Gleason grade and cancer aggressiveness. Our group recently used pSurv to mediate reporter gene expression on non-viral gene vectors called to minicircles (MCs), shortened plasmids stripped of prokaryotic components. In this work, we explore two iterations of these tumour-activatable minicircles (TA-MC) for PCa: (1) Diagnostic TA-MCs expressing Gaussia luciferase (GLuc), a sensitive reporter protein detectable in urine, and (2) Therapeutic TA-MCs encoding a fusion enzyme called cytosine deaminase uracil phosphoribosyl transferase (CD:UPRT) which metabolizes the prodrug 5-fluorocytosine (5-FC). We hypothesized being able to detect PCa by measuring urine GLuc encoded on diagnostic TA-MCs and attenuating growth of aggressive, high-survivin tumours using therapeutic TA-MCs in mice.

Materials and Methods: We constructed parental plasmids that expressed GLuc or CD:UPRT downstream of pSurv. TA-MCs were then made from these parental plasmids using a previously described production system. We used high-survivin PC3MLN4 and low-survivin LNCaP PCa cells to establish orthotopic tumours in nude mice and performed intratumoural injections with either diagnostic or therapeutic TA-MCs complexed with a linear polyethyleneimine. Urine samples were collected from mice receiving diagnostic TA-MCs, and GLuc activity was quantified using a commercial kit. For mice receiving therapeutic TA-MCs, PC3MLN4 tumour burden over 14 days was assessed using bioluminescence imaging and compared to sham mice that received saline instead of TA-MCs. Daily intraperitoneal 5-FC was administered to both sham and TA-MC treated mice.

Results: We found that urine GLuc was positively related to tumour survivin expression, being able to specifically discern mice with aggressive PC3MLN4 tumours from mice with non-aggressive LNCaP tumours and tumour-free mice. Administration of therapeutic TA-MCs to PC3MLN4 tumours resulted in reduced tumour burden in treated mice compared to sham mice at endpoint.

Discussion: Our work here is the first to encode cancer-inducible luciferase and therapeutic systems on minicircle vectors. Used sequentially or together, these TA-MCs could provide a clinically-relevant, tumour-specific system to identify and treat patients with aggressive PCa. Ultimately, we envision combining GLuc and CD:UPRT on the same TA-MC, creating a theranostic agent for simultaneous cancer detection and therapy.