Non-viral DNA-delivery system for familial adenomatous polyposis therapy in a rat model of human colon cancer

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Background
Familial adenomatous polyposis (FAP) is a heterozygous dominant inherited disease causing early onset colorectal cancer (CRC) in 100% of patients. A mutated version of the adenomatous polyposis coli (APC) gene is present in FAP patients, and within 60-80% of CRC cases. In FAP, benign polyps begin formation during the late teen years and frequently progress to carcinomas in patients by their early thirties, resulting in resection of the colon and duodenal segments.

Hypothesis
Recent studies in model systems have shown that re-established APC expression can induce CRC regression. We hypothesized that a functional APC gene replacement will lead to existing polyp regression and prevent adenomagenesis.

The F344-Apcmin/117c/PirC rat model
A mutation in the APC Gene characterizes the PirC rat model. Phenotypically, the model spontaneously develops polyps throughout the colorectum, and in later stages, small intestinal regions. The polyps follow a similar progression to those affected with FAP (Figure 1).

DNA-Lite Delivery Vehicle
DNA-Lite is a proprietary stem cell reaching epimodal based gene delivery vehicle designed by Andrew Lee and Timothy Dae. In vitro transfection of colon cancer lines show efficient introduction of the control GFP vector (Figure 2). It has been demonstrated by flow cytometry (Figure 3) that DNA-Lite transfects cells in vitro with 25% efficiency compared to Lipofectamine (Lipo), another liposomal based delivery vehicle. Unlike Lipofectamine, DNA-Lite is able to effectively penetrate through mucous to reach crypt cells. This makes it of interest in colorectal gene therapy.

Trial Design
Rats ranging from 120-210 days were divided into 3 groups (n=5), viz. Lipofectamine+APC (positive control), DNALite+APC (test group) and DNALite+GFP (to monitor the efficiency of the DNA-Lite system). The rats are anesthetized with isoflurane and a 0.5-1.0 mL PBS enema is given. After which, 750 µl of the select vehicle is delivered to the distal colon via rectal gauge. Tumor progression is monitored longitudinally throughout the trial by colonoscopies scheduled fortnightly. Blood draws are taken in parallel to monitor changes due to treatment.

Development of an in vivo measurement system
Eliminating subjectivity in longitudinal polyp monitoring is a challenge as objective methods in polyp sizing are confounded by the nature of colonoscopies. Using a known object to set a scale and further extrapolate the size of its surroundings has shown some consistency, but is labour intensive and often requires additional software. Here we set out to create a pixel to mm conversion to find cross sectional area of the tumour based on the images and a known distance.

Conclusions and future directions
• Visible expression of green fluorescence in the DNA-Lite+GFP group suggest transduction by the DNA-Lite delivery system reaching the crypt base and tumor cells.
• Tumor number and cross-sectional area will be monitored longitudinally via colonoscopy and our developed measurement methodology. We are particularly interested in how tumor changes compare between the DNA-Lite+APC and Lipofectamine+APC groups.
• Initial analysis of polyp cross-sectional area indicate successful APC gene replacement by the DNA-Lite delivery vehicle. The regression in polyp size is promising, especially when compared to the negative control group (DNALite+GFP) that maintained polyp size.
• At 240 days, post-mortem tumor size will be assessed grossly and histologically, and GFP uptake will be visualized through immunohistochemistry. Wet weights of the polyps will also be compared across cohorts.
• Upon completion of the trial, we expect a greater reduction of adenoma size in the DNA-Lite+APC group compared to the others.
• If successful, the DNA-Lite delivery system may provide gene therapy options for FAP patients to improve and extend quality of life in this often-debilitating disease.

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