COLONIC CYCLORAMAS AND SYNCHROTRON X-RAY PHASE CONTRAST MICRO-COMPUTED TOMOGRAPHIC IMAGING EMPLOYED TO STYDY COLORECTAL CANCER IN A MOUSE MODEL

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INTRODUCTION

Colorectal cancer (CRC) is the third most lethal cancer worldwide and the second in Europe [1]. Aberrant crypt foci (ACF), the earliest microstructural signs of CRC development on the colonic lumen [2, 3], are identified through advanced endoscopic techniques [3] and the existence of pathology is subsequently validated through standard histology. However, the correlation of the morphology of ACF with microstructural details deeper in the tissue, has not been established yet [4-6].

We hypothesise that X-ray micro-computed tomographic (μCT) imaging could be employed to obtain high-fidelity 3D representations of the colonic mucosal structure and identify the underlying pathology in terms of microstructural abnormalities. To this end, we induced progressive stages of CRC using a mouse model for CRC. We then applied synchrotron X-ray phase-contrast micro-computed tomography to reveal the three-dimensional microstructure in murine colons.

METHODOLOGY

An azoxymethane and dextran sodium sulphate (AOM/DSS) model [7, 8] for inflammation-associated colorectal cancer was used to induce CRC on male C57BL/6J mice. As shown in Figure 1, 18 mice (AOM/DSS group) were treated with one injection of carcinogenic substance (AOM) at the beginning of the study and then up to three cycles of repeating inflammation and healing using 2.5% DSS for 7 days (to induce inflammation phase) followed by 14 days of distilled water (to induce healing phase). 18 mice were used as controls (DSS only n=6, AOM only n=6, untreated n=6). Colons from mice sacrificed at the end of each cycle on day 24, 45 and 66 represent three progressive stages of CRC. The samples were fixed in 10% neutral buffered formaldehyde and embedded in paraffin wax following standard histopathological procedures.

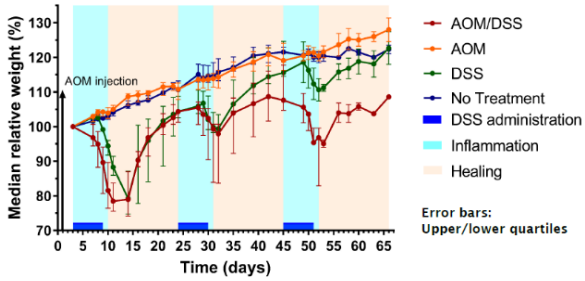

Figure 1: Whole body weight changes of male C57BL6/J mice using the AOM/DSS model. The graph shows the weight of each mouse relative to the beginning of the experiment as the median of each group. Mice sacrificed on day 24, 45 and 66 represent three progressive stages of CRC.

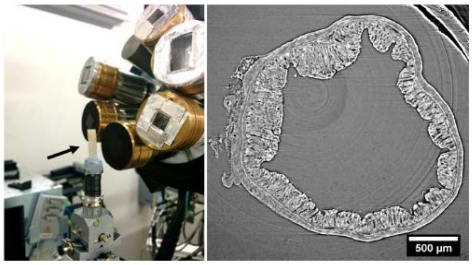

Figure 2: High resolution X-ray phase-contrast micro-computed tomography at beamline I13-2 of the Diamond Light Source. Left: Cylindrical wax blocks (arrow) on the sample holder. Right: Transverse CT slice of murine colon.

The resulting formalin-fixed paraffin embedded wax block samples were imaged with high-resolution X-ray phase-contrast micro-computed tomography (CT) at beamline I13-2 of the Diamond Light Source [9] (Photon energy: 25 keV, 2x binning, pixel size: 2.2 μm, number of projections: 2501). CT reconstruction was performed by applying standard filtered back-projection after Paganin phase retrieval [10]. As shown in Figure 2, this advanced and non-destructive imaging technique revealed the internal structure of the samples in the form of high-resolution image stacks, representing cross-sections of the colonic tube.

In order to study the structural evolution of the colonic crypts we developed an image processing technique that digitally unrolls these cross-sections. We create panoramic views of the gut (colonic cycloramas) as shown in Figure 3, which are essentially digital cross-sections of the crypts throughout several tissue layers, facilitating the understanding of the underlying pathology.

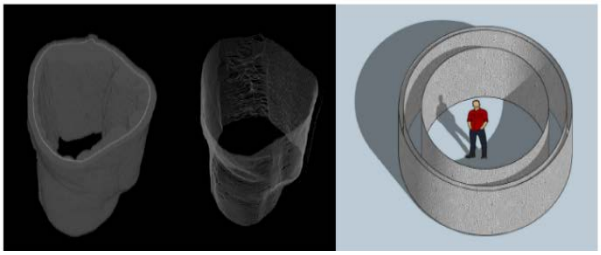

Figure 3: Colonic cycloramas. Left: A surface perpendicular to each crypt through the gut is defined at different tissue depths. Right: Photorealistic rendering of colonic cycloramas corresponding to three depths through the tissue layers.

CONCLUSIONS AND FUTURE WORK

We induced CRC to mice using an AOM/DSS model and non-destructively imaged the colonic samples in 3D through high-resolution synchrotron X-ray phase-contrast CT. High spatial resolution and image contrast allowed for resolving the micro-structural details of murine colonic crypts at different stages of CRC. We developed a novel image processing technique based on 3D data that advances the single-plane conventional histology by providing unprecedented colonic cycloramas. Each cyclorama reveals abnormal crypts and renders them visible and traceable throughout the whole colonic depth. These novel tools allow studying the microstructural changes during the early stages of CRC in 3D. We are currently using cycloramas to establish the correlation between the morphology of ACF on the mucosal surface and the structural abnormalities deeper in the tissue layers.

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