Space radiation affect the cell differentiation

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Abstract Space high energy Radiation is one of the major risks for astronauts. Our study was designed to delineate intestinal stem cell biological changes and gene expression signatures associated with space radiation. To investigate the biological effects of high-Z high energy (HZE) radiation in normal intestinal cells, we used RNA-Seq to characterize the transcriptomes of flow sorted cells from irradiated mice (0.5Gy $_{28}$Si) and controls. Distinct expression profiles were revealed in the stem cell versus non-stem cell compartments and in irradiated cells, 2 months after exposure, relative to controls. To further understand the differentiation of the stem cell under the radiation of Gamma ray and the response of adjacent tissues to the radiation, we further conduct the single cell RNA sequencing for the gene expression of each cell of the disassociation suspending cells. The mice subjected to the gamma radiation for two months, and then their intestinal tissues were disassociated and made for single suspending cells. The cells were used for constructed libraries and sequencing. Raw data were analyzed using cellranger pipeline on Biowulf2 and cellrangerRkit package on R-studio. We found that partial intestinal stem cell marker has expression on some intestinal cells. We got the gene list of each cluster in our TSNE plot and confirmed the cell type of each cluster on Enrichr website. Two different clusters of intestinal cell type were observed.

Introduction

The gene expression and differentiation affected by the low linear energy transfer radiation was reported widely in animal mode studies. However, in outer space, the radiation environment is variant. There are many high linear energy transfer energetic heavy ions such as ^{28}Si and ^{56}Fe whose effects on the human health during space explore remain unknown.[1]

Energetic heavy ions contribute significantly toward the dose equivalent of galactic cosmic radiation.It has been predicted that during a Mars mission about 30% of the astronauts' cells will be hit by either the primary or the secondary tracts of heavy ions[2]. The biological target damage and DNA damage induced by HZE radiation can directly affect the cell survival and genomic integrity of the cell. As more and more space explorations were conducted, the health risk for the people traveling in space need deep studied[3]. Gamma ray was studied as radiation exposure in our project.

The intestine exhibits remarkable regenerative potential, with intestinal stem cells (ISCs) residing in proliferative crypts and generating progenitors capable of multi-lineage differentiation and robust homeostatic and regenerative repopulation. The proliferative crypt zone contains an ISC ''niche'' composed of epithelial, subepithelial, and luminal components that provide essential paracrine signals[4]. Seminal studies defined Lgr5 as a molecular marker of crypt base columnar cells (CBC)-class ISCs that persist, self-renew, and generate all mature intestinal epithelial lineages in vivo[5]. CBCs can also be marked by Olfm4, notably Wnt-independent, and Ascl2, Prom1, Sox9lo, Cd24lo, Cd166, Grp78_, and Lrig1[6].

In this study used single cell sequencing technique to analyze the gene expression of the single stem cells and the adjacent cells affected by the gamma ray.

Methods

Mice: B6 mice and Lgr5-GFP mice get from Georgetown University. Only male mice were used as study subjects in this project. Mice were exposed to 0.5 Gy Gamma ray for 60 days. Then the intestinal tissues were harvested and digested by enzyme to get the single cell suspension in RPMI 1640 medium (Gibico). All animal experiments were conducted in accordance with procedures approved by the IACUC at National Institute of Health.
Single-cell RNA-SEQ: Single cell sequencing library construction used the GemCode platform. The single cell suspension concentration was controlled in 7X105~1.2X106 and designed to ensure the cell viability is over 70%. Then the cells were loaded in the GemCode Single Cell Instrument (10X Genomics, Pleasanton, CA) to generate single cell GEMs, approximately ~10000 cells were loaded per channel. Single cell RNA-seq libraries were prepared by GemCode Single cell 3' Gel Beads and Library Kit (#120237), Sequencing libraries were loaded at Illumina NextSeq500 with 2X75 paired-end Kits.
Alignment, barcode assignment and UMI counting: The cell Ranger single cell software suite was used to perform sample demultiplexing, barcode processing, and single cell 3' gene counting(https://support.10xgenomics.com/single-cell-gene expression/software/overview/welcome)
PCA and T-SNE analysis: We analyzed ten samples, the gene-cell barcode matrix was filtered based on number of genes detected per cell and percentage of mitochondrial UMI counts, the cells with less than 400 or more than 4,400 genes per cell were filtered, and the cells with more than 10% of mitochondrial UMI counts were filtered.

Results

After analysis one B6 intestinal tissue scRNA-seq sample, we found Lgr5 stems cells in the tissue and we further checked other stem cell molecular markers to confirm the stem cells (Figure 1). We further analyzed the gene expressions of the top ten genes in each cluster and plotted in Heatmap as in Figure 2.

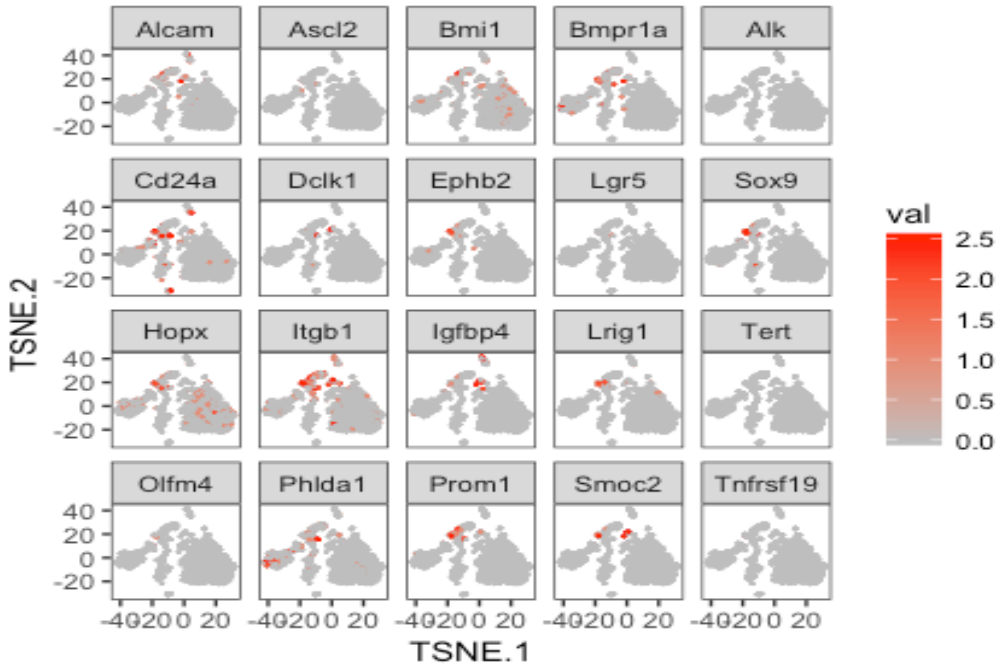

Figure 1 The stem cell marker exprssion in the clusters

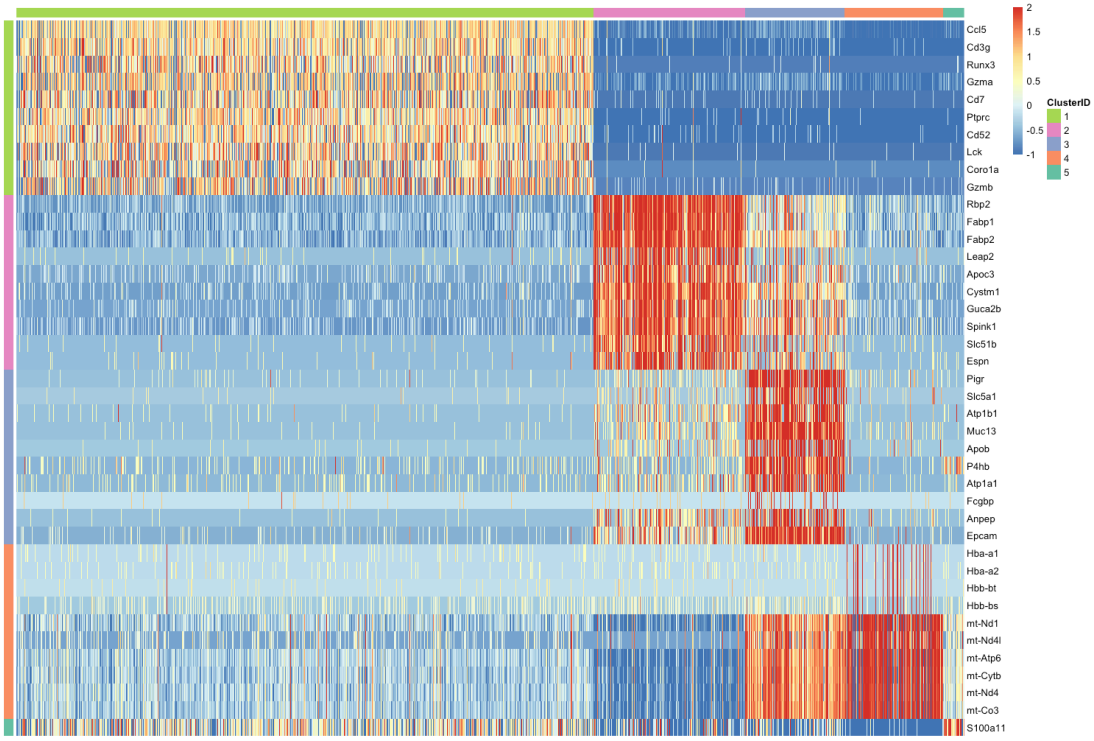

Figure 2 Expressions of the top ten genes in each cluster

Discussion

Although the number of stem cells locating in the intestinal tissue is low in our current study we did observe certain amount of stem cells in the intestinal tissue. Besides the current samples, another 9 samples (1.4 Gy ^{28}Si cases and controls) are under analyzed. For next step, we will merge the new fastq files results with the current data and enrich further data analysis.
In addition to stem cells, we also found numerous lymph cells in intestinal tissue. We may consider finding an appropriate way to remove these lymph cells before conducting library construction. There are still many work on proceeding for this project.

Reference

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