Abstract

Tumor organoids is a state of the art platform for precision medicine, especially more and more studies have shown that the treatment in organoids in vitro perfectly matches the patients’ response and tumor organoids could predict patients’ responses in clinics including colorectal cancer, pancreatic cancer, and ovarian cancer. Based on these studies, comparing the genetic data from the tumor organoids should be a strategy to investigate treatment resistance mechanisms and exploit new therapeutic target.

Methods: tumor organoids were obtained from our colorectal cancer organoids bank. RNA sequencing analysis was used to screen potential markers which play pivotal roles in mediating treatment response based on the organoids response to drug treatment and radiation. Candidate genes were analyzed by QPCR, and lenti-cas9 or dead-cas9-CRISPR technology was applied to investigate the gene function in cell lines and organoids.

Results: We found several differentially expressed genes between sensitive and resistant organoids groups which most are metabolism-related. Among them, GPX2 and FREM1 are found to tightly influence to 5-fluorouracil and irinotecan respectively on colorectal cancer cells, and both of them could increase radiation resistance. Overexpressing GPX2 could decrease cellular ROS levels, and increase stem cell marker CD24 level, while FREM1 could activate the NF-κB signaling and inhibit cell apoptosis induced by radiation and drug treatment.

Tumor organoids could be useful to explore new therapeutic targets in cancer treatment with higher precision. GPX2 and FREM1 which are upregulated in colorectal cancer increase cell proliferation and growth, cause radioreistance, higher GPX2 could enhance the IC50 of 5-fluorouracil while FREM1 increases the IC50 of irinotecan in colorectal cancer cells which imply this two molecules could be new therapeutic targets in the treatment of colorectal cancer.

Fig 1. GPX2 and FREM1 were highly expressed in the patients who responded poor to neoadjuvant therapy. The cluster analysis of differentially expressed gene in colorectal cancer organoids (A). Expression analysis of GPX2 and FREM1 in colorectal cancer tissues (B and C).

Fig 2. FREM1 had an oncogenic-like function in DLD-1. A FREM1 was overexpressed in DLD-1 cells through dead-cas9 crispr technology, its overexpression could activate NF-KB and ERK signaling. FREM1 overexpression prompted cell growth (B), enhance radioreistance (C) and inhibited cell apoptosis induced by radiation.

Fig 3. GPX2 contributed to 5-fluorouracil and radiation resistance. GPX2 was overexpressed in SW480 cancer cells (A), GPX2 could increase cell proliferation (B) and cause cells resistance to 5-fluorouracil (C) and radiation (D).

Fig 4. FREM1 and GPX2 increased the stem cell markers expression in colorectal cells. FREM1 overexpression enhanced CD24 and CD44 level in DLD-1 cells (A). GPX2 level was correlated with CD44 expression, as the GPX2 level was knocked down, CD44 also decrease significantly (C). Besides, GPX2 could help cancer cells GPX2 contributed to 5-fluorouracil and radiation resistance. GPX2 was eliminate cellular ROS level which could highly influence cells survival when cancer cells were treated with drugs and radiation (D).

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