Insights in Stem Cells

Extended Abstract
Vol. 6, Iss. 1
2020

Stem Cell Congress 2019-Doublecotin-Like Kinase 1 Increases Chemoresistance of Colorectal Cancer Cells through the Anti-Apoptosis Pathway- Lianna Li- Tougaloo College

Lianna Li[1], Kierra Jones[1] and Hao Mei[2]

[1]Biology Department, Tougaloo College, Tougaloo, USA, [2]Department of Data Science, University of Mississippi Medical Center, USA

Introduction

Colorectal Cancer (CRC) is the third most common cancer diagnosed in both men and women and the second leading cause of cancer-related deaths in the United States (http://www.cdc.gov/cancer/colorectal/statistics/). In the last 20 years, progress in the treatment of CRC has improved quality of patients' life, but up to 50% of patients relapsed aier surgical resection and ultimately died of metastatic disease. Adjuvant systemic chemotherapy with cytotoxic drugs is recommended as standard clinical practice for patients with stage III CRC aier surgical resection of the local CRC. since the survival outcomes of CRC patients with adjuvant systemic chemotherapy combined with surgical resection was significantly higher than those with surgical resection only. He promising progress of systemic chemotherapy for CRC began with the discovery of 5-fluorouracil (5- Fu) in 1957 [4]. Currently, the conventional first-line treatments for CRC patients are the combination of 5-Fu, leucovorin, and oxaliplatin (FOLFOX) or the combination of 5-Fu, leucovorin, and irinotecan(FOLFIRI) [5]. Recently, Curcumin was proven to be e ective in the inhibition of cell proliferation and migration of the chemoresistant CRC cells [6]. However, not all of the CRC patients respond to the systemic therapies, and even though for the responsive patients, almost all of them developed resistance [7]. According to the Cancer Stem Cell (CSC) hypothesis, the presence of chemoresistant CSCs (also known as Tumor Stem Cells (TSCs)) is the primary cause. CSCs accounts for 0.05% to 1% of the tumor mass, but they can give rise to all of the cell types in the tumor and possess unlimited selfrenewal capability. Several specific putative markers have been identified for the stem cell populations in the gastrointestinal tract, including doublecortin-like kinase 1 (DCLK1, also known as KIAA0369 or DCAMKL1. DCLK1 is a microtubule-associated serine-threonine protein kinase and functions in facilitating polymerization of tubulin dimers to assemble microtubules . It is predominantly expressed in the nervous system and is correlated with normal nervous system development and general cognition and verbal memory function. In the late 2000s, DCLK1 was identified as a stem cell marker for the intestinal stem cells and correlated with stemness of CRC cells . It is co-localized with other well-characterized gastrointestinal stem cell markers, such as Lgr5 in the "+4 position" of the crypt of the small intestine where the intestinal stem cells are located . Upregulated expression of DCLK1 was found broadly in solid tumors almost all over the body, including esophageal cancer, pancreatic cancer, liver cancer, CRC, etc. and is correlated with poor prognosis. He most recent clinical findings identified that elevated DCLK1+ cells in the blood can be used as a novel non-invasive marker for the diagnosis of incidence, relapse, and metastasis for CRC, liver cancer, pancreatic cancer, and Barrett's esophagus and esophageal adenocarcinoma . DCLK1 played important roles in the initiation, progression, and metastasis of CRC. It can promote cell survival via the prevention of cancer cell apoptosis in neuroblastoma and anoikis in mouse colonic epithelial cells . Hough DCLK1 is such multiple functional proteins in the CRC tumorigenesis, neither association of DCLK1 with chemoresistance in human CRC nor the underlying cellular and molecular mechanism is clear. In this paper, we identified that DCLK1 can significantly increase chemoresistance of CRC cells to 5-Fu treatment, and it functions through inhibition of gene expression of key caspases and activation of the apoptosis pathway. Our results demonstrated that DCLK1 can be used as an intriguing therapeutic target for CRC treatment.

Material and Methods

Cell line and cell culture

This work is partly presented at 2nd International Conference on Stem Cells and Regenerative Medicine May 20-21, 2019 held in Rome, Italy

Human colorectal carcinoma cell line HCT116 cells were purchased from ATCC (ATCC® CCL-247™) and were maintained in McCoy's 5A medium (ATCC® 30-2007™) supplemented with 10% FBS in 37°C incubator with 5% CO2 . Isogenic DCLK1 over-expressed cells (DCLK1+) were established by transfecting human DCLK1 variant 1 cDNA, which is fused with a turboGFP gene at C-terminal (OriGene, Cat #RG217050) into HCT116 cells. In order to avoid the clonal variance, different DCLK1 over-expressed clones were selected. Control HCT116 cells (WT) were established by transfecting pCMV6- AC-GFP Tagged Cloning Vector (Origene, Cat #PS100010) into HCT116 cells. Both DCLK1 over-expressed cells and control HCT116 cells were selected (400 µg/ml) and maintained (250 µg/ml) using Geneticin (G418). 5-Fu cytotoxicity assay WT and DCLK1+ cells were plated at 1 × 10^4 cells/well/100 µL in the 96-well plate for 24 hours. Hen cells were treated with 5-Fu (Sigma; F6627-1G) at different concentrations with 8 wells per dose concentration for 24 or 48 hours. Cell viability was determined by MTT assay according to Li's approach with modifications [36]. Briefly, MTT reagent (5 mg/ml) was added into cells at a 1:10 ratio of the culture medium and incubated for 3 hours at 37°C. Aier incubation, the culture medium with MTT was replaced by dimethyl sulfoxide (DMSO). He plate was sent to the BioTek Synergy 2 multi-mode reader and absorbance was measured at 570 nm and 630 nm. OD value used for cell viability calculation was calculated by subtracting OD630 (background) from OD570. Cell viability was determined by comparing the averaged calculated OD of 5-Fu treated cells to the DMSO-treated control cells. IC50 of 5-Fu was calculated from equation generated using Excel by the cell viability and dose-killing data. Briefly, select the data, insert charts and select "scatter plot". Hen set the Y-axis to "logarithmic", select "add trend line" and for the trendline options, select "exponential" and "display equation on chart". Using the equation, you can calculate the IC50. Western blotting WT and DCLK1+ cells were plated at 2 × 10^6 cells per T-25 flask and cultured for 24 hours. Hen cells were treated with/without 5-Fu and cultured for 48 more hours. Whole cell lysates were harvested using ice-cold RIPA buffer with 1X protease inhibitor (Sigma, P8340) and 1X phosphate inhibitor (Sigma, P5726). Protein concentration was determined using Pierce™ BCA Protein Assay Kit according to the manufacture's manual

References

1. De Dosso S, Sessa C, Saletti P (2009) Adjuvant therapy for colon cancer: present and perspectives. Cancer Treat Rev 35: 160-166. [PubMed]

2. Engstrom PF, Arnoletti JP, Benson AB, Chen YJ, Choti MA, et al. (2009) NCCN clinical practice guidelines in oncology: Rectal cancer. J Nat Compr Cancer Net 7: 838-881. [PubMed]

3. Wilkinson NW, Yothers G, Lopa S, Costantino JP, Petrelli NJ, et al. (2010) Long-term survival results of surgery alone versus surgery plus 5- fluorouracil and leucovorin for stage II and stage III colon cancer: pooled analysis of NSABP C-01 through C-05: A baseline from which to compare modern adjuvant trials. Ann Sur Oncol 17: 959-966. [PubMed]

4. Heidelberger C, Chaudhuri NK, Danneberg P, Mooren D, Griesbach L, et al. (1957) Fluorinated pyrimidines, a new class of tumor-inhibitory compounds. Nature 179: 663-666. [PubMed]

5. Lenz HJ (2008) First-line combination treatment of colorectal cancer with hepatic metastases: Choosing a targeted agent. Cancer Treat Rev 34: 3-7. [PubMed]

6. Hosseini SA, Zand H, Cheraghpour M (2019) He influence of curcumin on the downregulation of MYC, Insulin, and IGF-1 Receptors: A possible mechanism underlying the anti-growth and anti-migration in chemoresistant colorectal cancer cells. Medicina (Kaunas) 55: 1-12. [PubMed]

7. Dallas NA, Xia L, Fan F, Gray MJ, Gaur P, et al. (2009) Chemoresistant colorectal cancer cells, the cancer stem cell phenotype, and increased sensitivity to insulin-like growth factor-I receptor inhibition. Cancer Res 69: 1951-1957. [PubMed]

8. Dean M, Fojo T, Bates S (2005) Tumour stem cells and drug resistance. Nat Rev Cancer 5: 275-284. [PubMed]

9. Zeuner A, Todaro M, Stassi G, De Maria R (2014) Colorectal cancer stem cells: From the crypt to the clinic. Cell Stem Cell 15: 692-705. [PubMed]

This work is partly presented at 2nd International Conference on Stem Cells and Regenerative Medicine May 20-21, 2019 held in Rome, Italy

10. Yamakage K, Omori Y, Piccoli C, Yamasaki H (1998) Growth control of 3T3 fibroblast cell lines established from connexin 43-deficient mice. Mol Carcinog 23: 121-128. [PubMed]

lli@tougaloo.edu