Preclinical Evaluation of the Novel Small-Molecule MSI-N1014 for Treating Drug-Resistant Colon Cancer via the LGR5/β-catenin/miR-142-3p Network and Reducing Cancer-Associated Fibroblast Transformation

Vijesh Kumar Yadav

The Program for Translational Medicine, Graduate Institute of Biomedical Informatics, College of Medical Science and Technology, Taipei Medical University, Taipei 11031, Taiwan

Abstract

Colorectal cancer represents one of the most prevalent malignancies globally, with an estimated 140,000 new cases in the United States alone in 2019. Despite advancements in interventions, drug resistance occurs in virtually all patients diagnosed with late stages of colon cancer. Amplified epidermal growth factor receptor (EGFR) signaling is one of the most prevalent oncogenic drivers in patients and induces increased Janus kinase (JAK)/signal transduction and activator of transcription (STAT) and β-catenin functions, all of which facilitate disease progression. Equally important, cancer-associated fibroblasts (CAFs) transformed by cancer cells within the tumor microenvironment (TME) further facilitate malignancy by secreting interleukin (IL)-6 and augmenting STAT3 signaling in colon cancer cells and promoting the generation of cancer stem-like cells (CSCs). Based on these premises, single-targeted therapeutics have proven ineffective for treating malignant colon cancer, and alternative multiple-targeting agents should be explored. Herein, we synthesized a tetracyclic heterocyclic azathioxanthone, MSI-N1014, and demonstrated its therapeutic potential both in vitro and in vivo. First, we used a co-culture system to demonstrate that colon cancer cells co-cultured with CAFs resulted in heightened 5-fluorouracil (5-FU) resistance and tumor sphere-forming ability and increased side populations, accompanied by elevated expression of cluster of differentiation 44 (CD44), β-catenin, leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5), and ATP-binding cassette super-family G member 2 (ABCG2).

Keywords: **colon cancer**; **drug resistance**; **small-molecule therapeutics**; **cancer-associated fibroblasts (CAFs)**; **cancer stemness**; **miR-142**

Introduction

Colorectal cancer ranks as one of the most prevalent gastrointestinal cancer types globally, accounting for an estimated nine percent of all cancer cases [1]. Patients with advanced stages of CRC often manifest unfavorable phenotypes, including treatment resistance and distant metastasis, which lead to limited therapeutic options. Studies have shown that the tumor microenvironment plays a key role in the development and progression of CRC. One of the major components of the CRC TME is stromal fibroblasts. A subpopulation of these fibroblasts is referred to as cancer-associated fibroblasts which are closely linked to the malignant characteristics of late-stage CRC [2]. CAFs are characterized by the expression of alpha-smooth muscle actin (α-SMA), which promotes the metastatic potential and stemness within the TME [3]. CAFs contribute to the progression of CRC by secreting oncogenic cytokines such as interleukin (IL)-6, transforming growth factor (TGF)-β1, and epidermal growth factor (EGF), all of which are documented to promote the epithelial-to-mesenchymal transition (EMT) and induce stemness.

Materials and Methods

4.1. Cell Culture and Reagents

The DLD1 and HCT116 human colon cancer cell lines and normal fibroblasts were obtained from American Type Culture Collection and were cultured according to the vendor's recommended conditions. MSI-N1014 was synthesized as described previously in a U.S. patent application [12] (H.S. Huang, D.S. Yu, T.C. Chen, Vol. US Patent No. 8,927,717B1, US, 6 January 2015). Fluorouracil (5-FU) was purchased from SelleckChem (Hsinchu, Taiwan) (cat. no. S1209). Stock solutions of 5-FU and MSI-N1014 were dissolved in 10 mM dimethyl sulfoxide (DMSO; Sigma Aldrich, St. Louis, MO, USA) and kept at −20 °C. The stock solution was further immediately diluted in the sterile medium at the required concentrations.

4.2. Colon Tumor Sphere-Formation Assay

The tumor sphere-formation assay was performed according to a previously described method [35] with modifications. In short, colon cancer cells were seeded (2000 cells/well) in six-well ultra-low attachment plates (Corning, Corning, NY, USA) in serum-free media consisting of Dulbecco's modified Eagle medium /Ham's F12 (1:1), human epidermal growth factor basic fibroblast growth factor, 2 μg/mL 0.2% heparin and 1% penicillin/streptomycin (P/S, 100 U/mL, Hyclone, Logan, UT, USA).

Results

2.1. CAFs Increased Oncogenic Properties of CRC Cells with Increased Association of EGFR

CAFs were implicated in the development and progression of CRC. Herein, we demonstrated the tumor-promoting roles of CAFs, where we co-cultured DLD1 and HCT116 CRC cells with CAFs (Insert, **Figure 1**A). First, we demonstrated that CAF-educated DLD1 and HCT116 cells are more resistant against 5-FU (**Figure 1**A). For instance, under the influence of CAFs, the IC_{50} value of 5-FU of DLD1 cells was approximately 2-fold higher than its naïve counterpart. Second, our flow cytometric analysis showed that CAF

educated DLD1 and HCT116 cells showed increased percentages of the cluster of differentiation 44-positive cell population (Figure 1B), and more importantly, side-population (SP) cells (Figure 1C). These observations were accompanied by increased colony-forming (Figure 1D), migratory (Figure 1E), and self-renewal abilities (Figure 1F), compared to their naïve counterparts.

Discussion

TMEs have recently garnered increased attention in the development and progression of tumor cells [18]. The presence of CAFs was identified in many cancer types, including colon cancer, and they were indicated to contribute to the development of malignant properties such as distant metastasis and drug resistance [19,20]. Thus, it is no longer sufficient to target cancer cells when it comes to developing therapeutics. Drugs that have the ability to suppress both tumor-promoting signaling and CAF generation will definitely provide superior therapeutic effects compared to traditional targeted therapeutic compounds.

The present study first provides strong evidence that CAFs promote colon tumorigenic properties when co-cultured, establishing a drug screening platform. It was clear that CAF-educated DLD1 and HCT116 cells were significantly more tumorigenic, as featured by the increased abilities to resist 5-FU, form colonies, and tumor-spheres, and migrate; these increased tumorigenic properties were associated with increased expressions of LGR5, β-catenin, and mTOR signaling. In support, previous studies indicated that increased LGR5 expression was identified in drug-resistant colon cancer cells and was a key player in generating/maintaining colon cancer stem cells [21,22].

Conclusions

In summary, we provide preclinical evidence to support the therapeutic functions of MSI-N1014. MSI-N1014 was shown to function in suppressing the colon cancer stemness markers, LGR5, and β-catenin, while also preventing the transformation of CAFs. In part, MSI-N1014-mediated antitumor effects acted through the induction of the miR-142-3p tumor suppressor. Further investigation is warranted for developing MSN-N1014 as a therapeutic agent.

vijeshp2@gmail.com

This work is partly presented at Joint Event on 24th World Congress on Pharmacology & 7th World Heart Congress, August19- 20, 2019 at Vienna, Austria