A rationale for treatment of colorectal cancer with mitomycin C and crizotinib

Avital Lev[1], Elena Shagisultanova[2], Safoora Deihimi[1], David T. Dicker[1], Joanne Xui[3] and Wafik S. El-Deiry[1].

[1]Fox Chase Cancer Center, Philadelphia, PA; [2]University of Colorado Denver, CO and [3]Caris Life Sciences, Phoenix, AZ

Abstract

Colorectal cancer (CRC) is the third leading cause of cancer-related death in men and women in the United States. Microsatellite instability (MSI) is found in approximately 15% of sporadic colorectal cancers and in the majority of Lynch syndrome patients. MSI has been correlated to deficiency in the mismatch repair (MMR) genes and therefore MSI-High tumors exhibit higher mutation rates then non-MSI-High tumors. We recently reported on 26 MSI-High and 558 non-MSI-High CRCs that were profiled at Caris Life Sciences (Shagisultanova et al., 2015 ASCO Annual Meeting Abstract No. e14684 "Association of increase in BRCA2 gene mutations in microsatellite instable (MSI-H) colorectal cancer (CRC) with increased c-MET expression."). Immunohistochemistry and genomic analyses were performed in the BRCA-mutant versus BRCA wild-type MSI-High tumors. BRCA2 mutations were highly enriched (50%) in MSI-High CRC. MSI-High tumors with BRCA2 frameshift mutations had high c-MET expression. c-MET overexpression is known to be associated with aggressive metastatic CRC. We hypothesized there may be a synergistic drug interaction between drugs that are used to treat BRCA2-deficient tumors and c-MET inhibitors. We further hypothesized there may be a mechanistic link between BRCA2-deficiency, double-strand breaks following DNA damage, and c-MET overexpression. Mitomycin C (MMC) is an anti-cancer chemotherapeutic agent, which causes DNA damage by inducing double strand breaks (DSBs) through DNA cross-linking. Tumors deficient in genes encoding for proteins involved in DNA repair such as BRCA2 show hypersensitivity to MMC. Crizotinib is a small molecule inhibitor of c-MET and ALK receptor tyrosine kinases. In the present studies, we tested CRC cell lines for sensitivity to MMC plus crizotinib. CRC cell lines treated with MMC activated a DNA damage response as measured by up-regulation of γH2AX. Upon BRCA2 siRNA-mediated knockdown colorectal cancer cells became more sensitive to MMC shown by cleaved-PARP as a measure of apoptosis. Crizotinib inhibited the activation of c-MET in CRC cell lines treated with Hepatocyte Growth Factor (HGF). The combination treatment of colorectal cancer cells with crizotinib and MMC led to increased apoptosis (cleaved-PARP) compared to each drug alone. Combination treatment with increasing concentrations of both drugs in a CellTiter-Glo Cell Viability assay demonstrated a synergistic effect. However, we found no evidence for c-MET up-regulation upon effective BRCA2 knockdown in the absence or presence of DNA damage. Although there is no mechanistic link between BRCA2 mutation and c-MET overexpression, c-MET is frequently overexpressed in CRC in general and BRCA2 is frequently mutated in CRC especially in MSI-High cases. The combination of crizotinib with MMC appeared synergistic regardless of whether CRC cell lines were MSI-High or non-MSI-High. Our results prompt the clinical testing of the combination of MMC and crizotinib in advanced CRC.

Introduction

- Colorectal cancer (CRC) is the second leading cause of cancer related death in the United States for cancer of men and woman.
- Crizotinib is a small molecule inhibitor of c-MET, ALK and ROS1 receptor tyrosine kinases. It is FDA approved for NSCLC cancer expressing ALK or ROS1.
- Overexpression of c-MET and its kinase activity have shown to be a leading cause of tumor growth and metastasis in CRC.
- Mitomycin C (MMC) is an anti-cancer chemotherapeutic agent, which causes DNA damage by inducing double strand breaks (DSBs) through DNA cross-linking.
- Tumors deficient in BRCA genes show hypersensitivity to MMC.
- In this study we develop a rationale for combination treatment of advanced CRC patients with c-MET inhibitor crizotinib and MMC

Results

BRCA mutation in MSI-H CRCs correlate with high c-MET expression

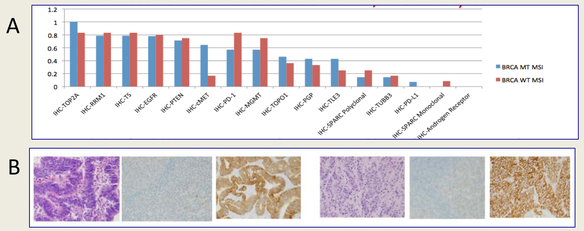


Figure 1 – A. 26 MSI-H and 558 non MSI-H were analyzed for BRCA 1/2 status and the expression of a panel of cancer markers by IHC. The comparison between BRCA mutant MSI to BRCA WT MSI samples revealed a correlation between mutated BRCA and high expression of the proto-oncogene c-MET. B. Representative IHC images of two cases of MSI-H BRCA mutant CRC with high c-MET expression.

BRCA2 Knockdown increases sensitivity of cells to MMC

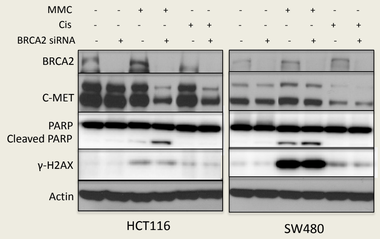


Figure 2 – Western blot analysis of HCT116 and SW480 CRC cell lines treated +/- MMC (5μg/ml) or cisplatin (5μM) for 24h following BRCA2 siRNA-mediated knockdown (KD). CRC cell lines treated with MMC activated a DNA damage response as measured by up-regulation of γ-H2AX. Upon BRCA2 siRNA-mediated knockdown colorectal cancer cells became more sensitive to MMC shown by cleaved-PARP as a measure of apoptosis. BRCA2 KD cells treated with MMC or cisplatin showed lower expression level of c-MET.

Crizotinib inhibits the activation of c-MET in CRC cell lines treated with Hepatocyte growth factor (HGF)

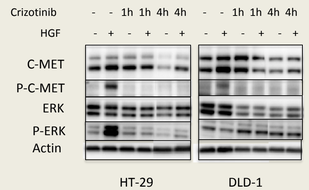
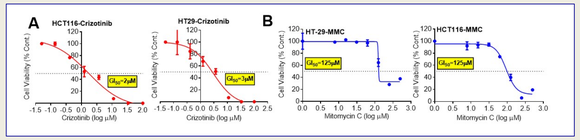


Figure 3 –HT-29 and DLD-1 CRC cell lines were treated with HGF. Activation of c-MET was then monitored by western blot analysis of phospho-c-MET. Treatment of the cells with crizotininb inhibited the phosphorilation of c-MET.

Results

Efficacy of CRC cell lines treated with single drug crizotinib or MMC



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Cell Line	MMC GI_{50}(μM)	Crizotinib GI_{50}(μM)
HT-29	125	3
HCT116	125	2
DLD-1	250	0.5
SW480	398	1.7

Figure 4– Representative dose response curves of HT-29 and HCT116 cell lines with crizotinib (A) and MMC(B). C. GI_{50} values (μM) were determined for both MMC and crizotinib on a panel of CRC cell lines.

Combination treatment of crizotinib and MMC increased apoptosis of CRC cells compared to each drug alone

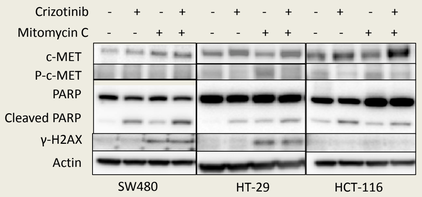

Figure 5 –Western blot analysis of CRC cell lines treated with +/- crizotinib or MMC or combination revealed increased level of apoptosis in the combination treatment compared to each drug alone. Apoptosis was measured by looking at PARP cleavage. Γ-H2AX levels were up-rgulated in samples treated with MMC.

Synergy between crizotinib and MMC in CRC cell lines

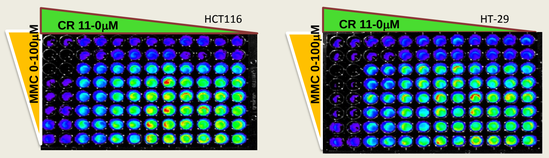

Figure 6– Synergistic potential of crizotinib and MMC was evaluated by cell titer-Glo assay.

Results

Crizotinib and MMC show potent synergistic potential in a CRC xenograft model

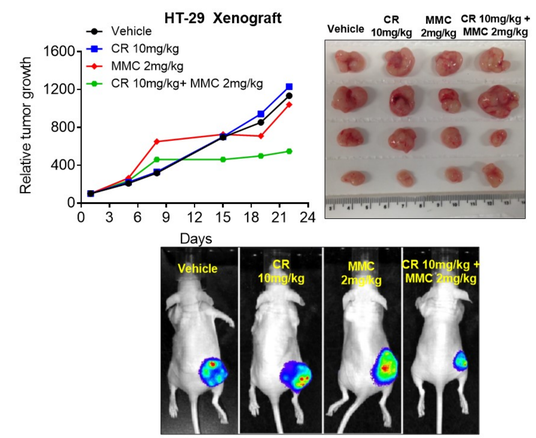

Figure 7 – HT-29-luciferase subcutaneous xenografted tumors were established in nude mice. Mice were then treated with 10mg/kg crizotinib oral gavage daily or with 2mg/kg MMC I.P. on day 1,4,7,10 and 13 or treated with combination of the 2 drugs. Biweekly measurements of tumor burden were taken with caliper measurement and bio-luminescence imaging of tumors was recorded weekly. A. Relative tumor growth curve shows inhibition of tumor growth in the group of mice that received the drug combination in comparison to each drug alone or vehicle group. B. Tumors on day 26 of treatment. C. Representative bio-luminescence imaging of 1 mouse per group at the last day of experiment.

Summary & Future Direction

- BRCA mutations in MSI-H CRC patients correlate with high c-MET expression
- BRCA2 KD increased the sensitivity of CRC cell lines to MMC
- Treatment of CRC cell lines with crizotinib inhibited the activation of c-MET by HGF
- Combination treatment of CRC cell lines with crizotinib and MMC induced greater apoptosis then either drug alone.
- Crizotinib and MMC drugs showed synergy in CRC cell lines *in-vitro*
- *In-vivo* xenograft model of CRC demonstrated the advantage of combination of crizotinib and MMC in inhibiting tumor growth compared to each drug alone.
- The proposed combination treatment of c-MET inhibitor and MMC will be further evaluated in additional CRC *in-vivo* models in order to provide a rationale for combination treatment for advanced CRC patients.