conclusion:
The aim of this study was to identify the relationship between MET amplification, protein or mRNA expression, and mutations in colorectal cancer (CRC). Our results showed that MET protein expression was higher in colorectal tumor tissue compared with adjacent normal intestinal epithelium. Positive MET protein expression was associated with significantly poorer overall survival (OS) and disease-free survival (DFS). Multivariate analysis revealed that positive MET protein expression was an independent risk factor for DFS, but not for OS. MET mRNA expression was upregulated in tumor tissues compared with the adjacent normal tissues. The incidence of MET amplification was 4.4%. None of the patients was positive for MET mutation. In our study, MET was overexpressed in colorectal adenocarcinoma, and its positive protein expression predicted a poorer outcome in CRC patients. Furthermore, according to our results, MET amplification and 14 exon mutation are extremely rare events in colorectal adenocarcinoma.

Introduction
Previous studies have reported overexpression, amplification, or mutation of MET as the most common ways of MET pathway dysregulation. In addition, it was reported that MET amplification is related to acquired resistance in patients without KRAS mutations during anti-EGFR therapy. Therefore, in order to better define an adequate target population and selection strategy for treatment with anti-EGFR therapy, it is also essential to understand the role of MET alterations in the acquired resistance. Based on these findings, our study was aimed to determine the relationship between MET amplification, protein and mRNA expression, as well as mutations in CRC.

MET protein expression of patients

Representative IHC staining images of MET protein expression in tissue microarrays: (A) Negative MET expression in colonic epithelium, (B) negative MET expression, (C) positive cytoplasm staining and (D) positive membranous staining in colorectal adenocarcinoma. Original magnification × 100 (× 200 for insert).

MET Amplification of patients

Representative images of MET protein expression examined by FISH. (A) Negative, (B) positive FISH with a GCN of 6.4, and (C) positive FISH with a MET/CEP ratio of 2.62. FISH: fluorescence in situ hybridization; GCN: gene copy number. Original magnification × 400 in oil.

MET mRNA expression of patients and survival analysis

Ectopic expression of MET predicted poor survival in colorectal adenocarcinoma patients. (A) Expression of MET in the TCGA colorectal cancer RNAseq dataset (normal n=51, tumor n=647), (B) Expression of MET in the FDUSCC dataset (normal n=61, tumor n=72), (C) Kaplan-Meier curves for CRC patients OS in the TCGA dataset (n=617), (D-G) Kaplan-Meier analyses for the FDUSCC dataset. (D) OS and (E) DFS according to MET mRNA expression. (F) OS and (G) DFS according to MET IHC score. (H) OS and (I) DFS according to MET FISH results. OS overall survival, DFS disease free survival.

Association between MET expression, MET amplification and MET mRNA expression

(A-C) Spearman’s correlation analyses between MET H score, mRNA level, and GCN of FISH in 72/294 colorectal adenocarcinoma tissues.

In the future, we will continue to find the new therapy target of CRC, and will focus on the new strategy for treatment of anti-PD1 and EGFR therapy.