Quantitative immunofluorescence assessment of MET and epithelial-to-mesenchymal transition (EMT) biomarker modulation by antiangiogenic inhibitors in xenograft tumor tissues

Tony Nase1, Scott M. Lawrence1, Donna Butcher1, Lindsay M. Dutko1, Melissa G. Hollingshead1, Robert L. Kinder1, Ralph E. Parchment1, Donald P. Bottaro2, W. Marston Linehan3, Joseph E. Tomaszewski4, Apurva K. Srivastava4, and James H. Doroshow5

1Lilly Laboratory of Human Technology and Pharmacology, Applied/Developmental Research Directorate, Leidos Biomedical Research, Inc., 2Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702; 3Pathology/Technology Laboratory, Laboratory Animal Sciences Program, Leidos Biomedical Research, Inc.; 4Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702; 5Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702; 6Toxicology Testing Branch, Developmental Therapeutics Program, National Cancer Institute, Frederick, MD 21702; 7Tissue of Cancer Treatment and Diagnosis and Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892

Introduction

Evaluation of MET inhibitors in xenograft tumor tissues revealed a complex relationship between MET expression and response to therapy. A potential explanation for this lack of concordance is that MET expression is not a static indicator of activity due to a dynamic balance of pro- and antiangiogenic signaling. In this study, we investigated whether antiangiogenic inhibitors induce a pro-MET survival signaling environment that enables MET expression to become enriched in tumor samples.

Methods

Two xenograft tumor models were used: one generated by subcutaneous injection of MKN45 cells in nude mice (the MKN45 xenograft model), and the other was a primary tumor xenograft model generated from a patient harboring an H1112R c-MET kinase mutation. Xenografts were treated with either vehicle, crizotinib (21), pazopanib (26), tivantinib (21), or a combination of crizotinib and pazopanib for 4 weeks. Patients treated with the combination had no activity on its own in inhibiting pY1235-MET. Figure 2A shows that xenograft samples stained with antibodies to MET (green) and total Vimentin (pink; primarily membrane localization) showed a mesenchymal phenotype in the treated MKN45 tumors. The EMT phenotype is shown as Log (V:E) > 0 and Epithelial phenotype (green) is expressed as Log (V:E) < 0. Left panel shows the presence of a mesenchymal phenotype in xenograft tumors treated with vehicle. Right panel shows that xenograft tumors treated with combination therapy for 4 weeks had a relative increase in expression of epithelial markers, shown as Log (V:E) < 0.

Results

Inhibitors Induced EMT

Quantitative assessment of pY1235-MET staining specifically using anti-MET and non-specific IgG as controls showed a significant increase in MET staining in tumor tissues treated with crizotinib, pazopanib, and the combination therapy compared to vehicle controls. Figure 3A shows the quantitation analysis of pY1235-MET staining in xenograft tumor tissues treated with vehicle or crizotinib (21). pY1235-MET staining was significantly increased in xenograft tumor tissue treated with crizotinib (21) compared to vehicle controls. Figure 3B shows a comparison of IFA vs. ELISA quantitation analysis of pY1235-MET staining in xenograft tumor tissues treated with vehicle or crizotinib (21). The ELISA assay showed a significant increase in pY1235-MET staining in xenograft tumor tissues treated with crizotinib (21) compared to vehicle controls.

Significance

The results presented in this study demonstrate that antiangiogenic inhibitors induce a pro-MET survival signaling environment that enables MET expression to become enriched in tumor samples. This finding suggests that MET expression is not a static indicator of activity due to a dynamic balance of pro- and antiangiogenic signaling. The identification of this dynamic relationship may provide new insights into the complex relationship between MET expression and response to MET-targeted therapies. These findings also have important implications for the development of MET-targeted therapies in the treatment of epithelial cancers.

References