Evaluation of HGF and MET protein expression in NSCLC tumor specimens from patients previously treated with targeted or chemotherapies

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Introduction

Although epidermal growth factor receptor (EGFR) TKI resistance accounts for majority of the resistance to the first-generation EGFR tyrosine kinase inhibitor (TKI), addition of MET (the HGF receptor) has also been identified as a resistance mechanism in patients (Badii and Bagla, 2013; Straussman et al., 2012; Wilson et al., 2012; Gurrieri et al., 2015). Effective treatment of patients that have progressed on first-generation TKI requires diagnostic assays that parallel the major resistance mechanisms and direct therapy selection. We have developed in vitro/in vivo [HGF] assays to detect both HGF and MET protein expression in formalin-fixed paraffin-embedded (FFPE) tissue, and evaluated expression levels in patients previously treated with EGFR/TKI chemotherapy.

Materials and Methods

HCC tissues were obtained from the Venture Biorepository. The assay was validated using the immunoprecipitated protein extracted from A549, HCC4856L, and Santa Cruz Biotechnology were evaluated using cell line pellets. Three of the five antibodies evaluated in this study (20X) were obtained from Santa Cruz Biotechnology and one from Biorvis Institute and used in validation of the selected HGF antibody (Figure 1). MET was both membranous and cytoplasmic staining was defined as positive. The expression was scored on the basis of intensity and fraction of positive cells. The intensity score was defined as follows: 0 = no observable staining in the tumor cells; 1 = faintly visible, weak membranous and cytoplasmic staining; 2 = weak to moderate staining; 3 = strong staining. Samples that scored 3+ in the slides of tumor were considered MET positive. MET IHC: Cytoplasmic staining was defined as positive. The expression was scored predominantly based on an intensity as defined for MET above. In addition, in-score was determined for the set of lung cancer specimens.

Figure 1: Evaluation of specificity of HGF IHC using cell line pellets.

Figure 2: Evaluation of sensitivity and specificity of HGF IHC using FFPE tissue.

Materials and Methods

IBL BladderLS – Gall (20X) IBL BladderLS – Gall (20X) IBL – Gall (20X) IBL – Gall (20X).

For cMMD of −2 and −3+ Met staining in the Left panels and HGF staining in the Right panels.

Table 1: cMet and HGF IHC data on 25 NSCLC specimens.

Table 2: cMet and HGF IHC data on 25 NSCLC specimens.

Conclusions

We have developed robust assay for evaluation of MET and HGF protein expression in FFPE sections and applied these assays in assessing NSCLC patient samples. The presented results show that MET and HGF expression are associated with resistance to targeted therapies. As beyond protein expression levels, will be needed to elucidate a relationship between MET pathway activation and response to EGFR TKIs.

References


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