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

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Abstract A42

Introduction

Although epidermal growth factor receptor (EGFR) T790M mutation accounts for majority of the resistance to the first generation EGFR tyrosine kinase inhibitors (TKIs), activation of the MET pathway through increased expression of hepatocyte growth factor (HGF) or amplification of MET (the HGF receptor) has also been identified as a resistance mechanism in patients (Sadig and Salgia, 2013; Straussman *et al.*, 2012; Wilson *et al.*, 2012; Corso *et al.*, 2013). Effective treatment of patients that have progressed on first-generation TKIs requires diagnostic assays that detect the major resistance mechanisms and direct therapy selection. We have developed immunohistochemistry (IHC) assays to detect both HGF and MET protein expression in formalin-fixed paraffin-embedded (FFPE) tissues, and evaluated expression levels in patients previously treated with EGFR TKIs or chemotherapy.

Materials and Methods

IHC assays were developed using the Ventana Benchmark. The MET (SP44) antibody was obtained from Ventana Medical Systems. The assay was validated using the recommended protocol supplied by the vendor. Five different HGF antibodies purchased from three vendors (IBL, LifeSpan Bio, and Santa Cruz Biotechnology) were evaluated using cell line pellets genetically engineered to over-express HGF as well as the parental cell line (Figure 1). FFPE human tissue specimens were obtained from ILSbio LLC, Asterand, Inc., BioServe, and a collaborator. In addition, a cancer tissue array with 48 cases of metastatic cancers from eight anatomical sites was purchased from BioChain Institute and used in validation of the selected HGF antibody (Figure 3).

MET IHC: Both membranous and cytoplasmic staining was defined as positive. The expression is scored on the basis of intensity and fraction of positive cells. The intensity score is defined as follows: 0 – no appreciable staining in the tumor cells; 1 – faint/barely visible partial membrane and cytoplasmic staining; 2 – weak to moderate staining; 3 – strong staining. Samples that scored ≥2+ in the ≥50% of tumor were considered c-Met positive.

HGF IHC: Cytoplasmic staining was defined as positive. The expression was scored predominantly based on intensity as defined for MET above. In addition, H-score was determined for the set of lung cancer specimens.

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

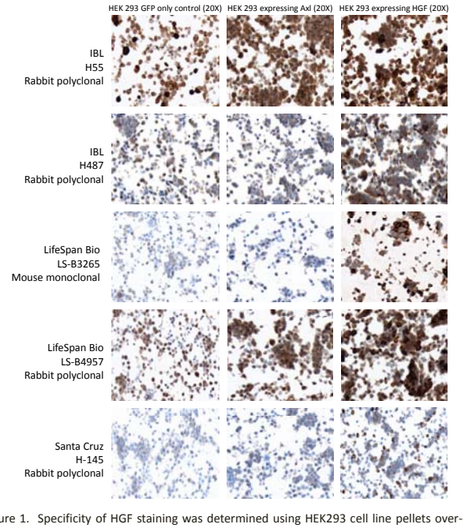

Figure 1. Specificity of HGF staining was determined using HEK293 cell line pellets over-expressing HGF cDNA (Right Panels). HEK293 parental cell line as well as cell line over-expressing Axl cDNA were used as negative controls (Left and Middle Panels). Three of the five antibodies evaluated (LS-B3265, IBL H487, and Santa Cruz H-145) showed differential staining of the HGF over-expressing cell lines compared to the parental or Axl over-expressing cell lines.

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

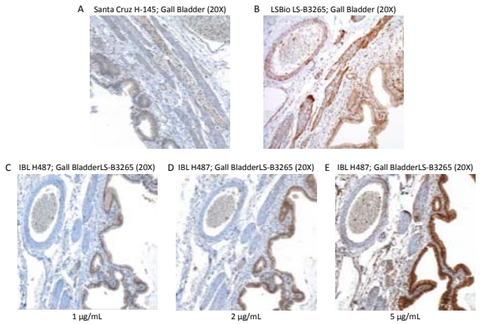

Figure 2. The three antibodies that displayed specificity for HGF over-expressing cell pellets were further evaluated for sensitivity and specificity using gall bladder FFPE tissue. Panels A-C display the staining patterns observed with the three antibodies. The IBL H487 antibody gave the best IHC signal compared to the other two antibodies based on intensity and specificity of the staining pattern. We tested several dilutions of the H487 antibody to determine the optimal concentration for further analysis (Panels C-E).

Figure 3: Representative HGF IHC images

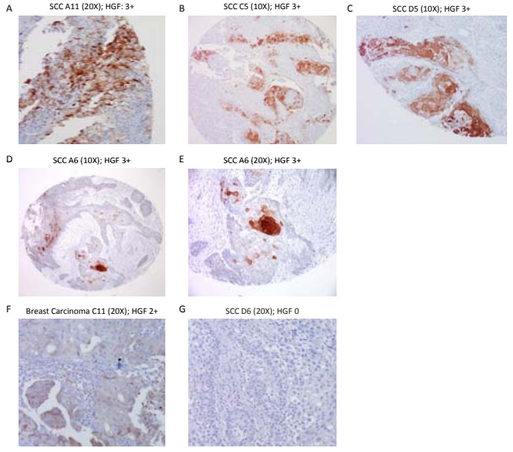

Figure 3. 48 metastatic tissues from 8 anatomical sites were evaluated for HGF expression. Panels A-G show representative images of 3+ to 0 scoring for HGF IHC, as indicated. Notably, in this set of specimens, it was primarily selected lung squamous cell carcinoma (SCC) specimens that showed robust 3+ HGF expression (Panels A-E; Panels D and E are the same specimen shown at different magnifications).

Figure 4: Representative c-Met IHC images

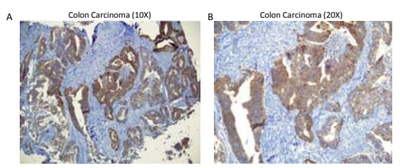

Figure 4. Representative example of a colon carcinoma specimen evaluated for c-Met expression. Panels A and B show a specimen with 3+ positive c-Met staining at 10X and 20X magnification, respectively.

Table 1: c-Met and HGF IHC data on 25 NSCLC specimens

ID	Tumor %	Stage/ Grade	Previous treatment history	c-Met Score	c-Met (+/-)	HGF score	HGF (H-score)
MMD-2-2-148	75%	IV [a]	TX1 Platinum-Based Doublet	0	Negative	weak positive 1+	10
MMD-2-2-149	80%	IV [a]	TX1 Platinum-Based Doublet	3+	positive-membranous	no tissue staining	no tissue staining
MMD-2-2-150	95%	IV [b]	newly dx	0	Negative	weak positive 2+	40
MMD-2-2-151	98%	IV	newly dx	3+	positive-membranous	negative	0
MMD-2-2-152	90%	IV [c]	TX1 Platinum-Based Doublet	3+	positive-membranous	weak positive 1+	70
MMD-2-2-153	60%	IV	newly dx	3+	positive-membranous	weak positive 2+	80
MMD-2-2-154	40%	IV [c]	newly dx	2+	Positive-cytoplasmic	negative	90-2 pieces of tx no staining
MMD-2-2-155	60%	IV	TX1 Platinum-Based Doublet	3+	positive-membranous	strong positive 3+	160
MMD-2-2-156	50%	IV [c]	TX1 Bevacizumab carboplatin, paclitaxel	0	Negative	negative	0-scant tumor left
MMD-2-2-157	85%	IV	Unknown	2+	positive-membranous	negative	0
MMD-2-2-158	75%	IIIA	Docetaxel Paraplatin	3+	positive-membranous	Strong positive 3+	115
MMD-2-2-002	75%	IIA/2	Unknown	3+	positive-membranous	weak positive 1+	20
MMD-2-2-005	80%	n.a./2	Unknown	3+	positive-membranous	strong positive 3+	140
MMD-2-2-006	70%	IIIA/2	Unknown	3+	positive-membranous	positive 3+	90
MMD-2-2-007	95%	IIB/3	Unknown	3+	positive-membranous	negative	0
MMD-2-2-010	85%	IIB/2	Unknown	3+	positive-membranous	weak positive 1+	70
MMD-2-2-013	95%	n.a./3	Unknown	2+	positive-membranous	negative	0
MMD-2-2-028	90%	IIB/3	Unknown	2+	positive-membranous	negative	0
MMD-2-2-030	95%	IB/2	Unknown	3+	positive-membranous	strong positive 3+	110
MMD-2-2-032	90%	IIB/1	Unknown	2+	positive-cytoplasmic	weak positive 1+	80
MMD-2-2-069	95%		Unknown	0	negative	negative	0
MMD-2-2-074	65%	n.a.	Unknown	0	negative	negative	0
MMD-2-2-075	85%	IIIA	Unknown	2+	positive-membranous	weak positive 1+	70
MMD-2-2-076	70%	IIIB	Unknown	0	negative	weak positive 1+	40
MMD-2-2-079	90%	IIIA	Unknown	3+	positive-membranous	negative	0

[a] lymph note metastasis
[b] pituitary metastasis
[c] metastasized tissue, site uncertain

Figure 5: Representative c-MET and HGF IHC images from NSCLC specimens

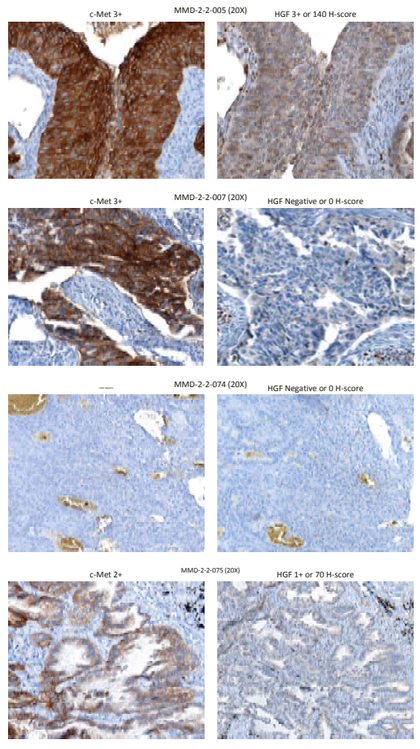

Table 1 and Figure 5. 25 NSCLC FFPE specimens were evaluated for both c-Met and HGF expression. 19/25 specimens tested showed positive c-MET expression; 13 were 3+ and 6 were 2+ in terms of staining intensity. 2 specimens that were 2+ score exhibited predominantly cytoplasmic staining. HGF expression was more variable with 5/24 exhibiting strong, 9/24 weak and 10/24 negative staining. There was no correlation between MET and HGF expression and patient status as relapsed versus newly diagnosed. Selected specimens are displayed in Figure 5, with c-Met staining in the Left Panels and HGF staining in the Right Panels.

Figure 6: Graphical representation of MET and HGF IHC data

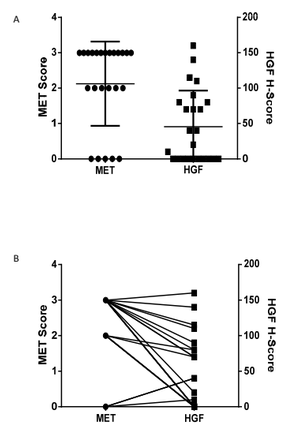

Figure 6. Panels A and B demonstrate the distribution of MET and HGF scores in the 25 FFPE specimens evaluated, and the correlation between the HGF and MET score.

Conclusions

We have developed robust assays for evaluation of MET and HGF protein expression in FFPE sections and applied these assays in analyzing 25 NSCLC patient specimens. A more expansive dataset, potentially evaluating factors beyond protein expression levels, will be needed to elucidate a relationship between MET pathway activation and response to EGFR TKIs.

References

Corso, S. and Giordano, S. 2013. *Cancer Discovery* 3(9):978-992.

Straussman, R. *et al.* 2012. *Nature* 487(7408):500-4

Wilson T.R. *et al.* 2012. *Nature* 487(7408):505-9

Sadiq A.A. and Salgia R. 2013. *J Clin. Oncol.* 31(8):1089-96

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