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Original contribution

c-MET immunohistochemistry for differentiating malignant mesothelioma from benign mesothelial proliferations☆,☆☆

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Summary The separation of benign from malignant mesothelial proliferations can be a difficult problem for the surgical pathologist. c-MET is a receptor tyrosine kinase that is overexpressed and detectable by immunohistochemistry in many malignancies, including malignant mesothelioma. Whether c-MET is also expressed in benign mesothelial reactions is unclear from the literature. To determine whether c-MET immunohistochemistry can separate benign from malignant mesothelial processes, we stained 2 tissue microarrays containing 33 reactive epithelioid mesothelial proliferations (E-RMPs), 23 reactive spindle cell mesothelial proliferations, 45 epithelioid malignant mesotheliomas (EMMs), and 26 sarcomatoid/desmoplastic mesotheliomas (SMMs) for c-MET and compared the results with immunohistochemistry for two established markers, BAP1 and methylthioadenosine phosphorylase (MTAP). Membrane staining for c-MET was evaluated using a 12-point H-score classified as negative (score = 0), trace (score = 1–3), moderate (score = 4–6), and strong (score = 8–12). Staining was seen in only 3 of 33 (all trace) E-RMPs compared with 36 of 45 (80%) EMMs (chi-square comparing reactive and malignant = 39.80, $p = 1.2 \times 10^{-8}$). The H-score was >3 (moderate or strong) in 24 of 45 (53%) EMMs. Addition of BAP1 staining to the c-MET–negative/trace EMM increased sensitivity to 75% (32/42), whereas similar addition of MTAP staining increased sensitivity to 77% (33/43). No benign spindle cell proliferations showed staining compared with 10 of 26 (38%) positive SMMs, but only 4 (15%) SMMs were classified as moderate or strong. We conclude that moderate/strong c-MET staining can be used to support a diagnosis of EMM vs an epithelial reactive proliferation. c-MET is too insensitive to use for detecting SMM.

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1. Introduction

The separation of benign from malignant mesothelial proliferations is often a difficult diagnostic problem. In many such cases, routine morphology is not helpful, and the pathologist is forced to depend on a variety of ancillary tests such as BAP1 and methylthioadenosine phosphorylase (MTAP) immunohistochemistry and *CDKN2A* (*p16*) fluorescence in situ hybridization (FISH) (reviewed in the study by Churg and Naso [1]). These tests are specific for malignancy when abnormal, but none of them is anywhere near 100% sensitive, even in combination.

c-MET is a receptor tyrosine kinase encoded by the *MET* proto-oncogene. When activated, normally by binding of its ligand, hepatocyte growth factor (HGF), c-MET signaling drives cell proliferation, motility, survival, and invasiveness [2–4]. c-MET plays an important role in embryonic development through a process called invasive growth [3,4] and in wound healing, but otherwise is usually expressed at a low level in normal tissues; however, expression is upregulated in a wide variety of malignancies wherein aberrant c-MET expression usurps the embryonic invasive growth pattern and instead contributes to tumor invasion and metastases [3–5]. One of the pathways through which c-MET signals are overexpressed is by phosphorylation of human epidermal growth factor receptor-3 (HER3, also called ERBB3) [6].

In biopsy series, immunohistochemical detection of c-MET has been reported in 74–100% of malignant mesotheliomas [7–10]. Whether c-MET is detectable by immunohistochemistry in benign mesothelial proliferations is unclear; the literature reports staining fractions ranging from 0% to 100% of such cases ([2], [9], [10], and see Discussion].

In this article, we examine immunohistochemical staining for c-MET in malignant mesotheliomas and benign mesothelial reactions to try to determine whether such staining can be used to separate benign from malignant mesothelial proliferations. We also investigate whether HER3 staining can be used for the same purpose.

2. Materials and methods

2.1. Case selection and tissue microarray preparation

The study was approved by the Research Ethics Review Board of the University of British Columbia and Vancouver Coastal Health (Vancouver, British Columbia, Canada). All mesothelioma cases were retrieved from the Vancouver General Hospital archive and confirmed by clinical findings, morphology, and immunohistochemical staining. All cases of reactive mesothelial proliferations were confirmed by clinical follow-up. Two tissue microarrays (TMAs) were constructed (core size: 0.6 mm, a minimum of two cores per case). In total, the two arrays contained 33 reactive epithelioid mesothelial proliferations (E-RMPs), 23 reactive spindle cell mesothelial proliferations (S-RMPs), 45 epithelioid malignant mesotheliomas (EMMs), and 26 sarcomatoid/desmoplastic mesotheliomas (SMMs).

2.2. Immunohistochemistry and scoring

Immunohistochemistry for c-MET was performed on a Dako Omnis (Aglilent, Santa Clara, CA) automated IHC instrument using Abcam (Aglilent, Santa Clara, CA) anti-MET (c-MET) rabbit monoclonal clone SP44 at a dilution of 1:50 with heat-induced epitope retrieval at low pH for 30 min and visualized using the Dako EnVision™ FLEX + detection system. BAP1 primary antibody (dilution: 1:50, SC-28383; Santa Cruz Biotechnology, Mississauga, Ontario, Canada) was used with heat-induced epitope retrieval at pH 9.0 for 30 min. The MTAP primary antibody (dilution: 1:100, 2G4; Abnova, Walnut Creek, California) was used with heat-induced epitope retrieval at pH 9.0 for 40 min. Immunohistochemical staining for HER3 was performed using anti-HER3 (catalog 12708; Cell Signaling Technologies, Beverly, MA) at a dilution of 1:50 with heat-induced epitope retrieval at pH 9.0 for 30 min.

c-MET was interpreted using an H-score system. For epithelial proliferations, we initially attempted to score both membranous and cytoplasmic staining; however, cytoplasmic staining was often much more heterogeneous and harder to interpret than membrane staining, so only membrane staining was formally evaluated. HER3 staining was scored in the same fashion as c-MET. Scoring was carried out by consensus between H.Z.R. and A.C. For spindle cell proliferations, we did not separate membrane from cytoplasmic staining because, while some spindle cells are plump enough to potentially distinguish the two, many spindle cells are so thin that this distinction is not possible.

We assigned two scores: a 5-tier scale for proportion of cells with positive staining as 0, 1–25%, 26–50%, 51–75%, and >75% (graded 0–4) and a 4-tier scale for intensity of staining (graded 0–3). The scores were multiplied providing a range of 0–12 which was subsequently grouped into negative, trace (score = 1–3), moderate (score = 4–6), and strong (score = 8–12; scores 5, 7, 10, and 11 do not exist in this scheme). BAP1 staining was assessed as nuclear loss or retention. MTAP was assessed for cytoplasmic loss or retention only [11].

2.3. Statistical analysis

Statistical significance between reactive mesothelial proliferation and mesothelioma was calculated using chi-square analysis. The sensitivity and specificity for c-Met in distinguishing between benign mesothelial proliferation and epithelioid mesothelioma and SMM was determined

using an online statistical tool (https://www.medcalc.org/calc/diagnostic_test.php).

3. Results

3.1. c-MET staining

Fig. 1 shows the combined staining from the two microarrays by individual H-score, and Table 1 provides these data broken down into H-score groups. Only 3 of 33 reactive epithelioid proliferations showed any staining for c-MET, and all 3 were scored as 2 (H-score group, trace) (Table 1 and Fig. 2, and see Discussion). In contrast, membrane staining was seen in 36 of 45 (80%) epithelioid mesotheliomas, and staining was scored as >3 (H-score groups, moderate or strong) in 24 of 45 (53%) epithelioid mesotheliomas (Table 1 and Fig. 2). The distribution of H-scores comparing reactive vs mesothelioma was statistically highly different (chi-square = 39.80, df = 3, $p = 1.2 \times 10^{-8}$).

None of the 23 reactive spindle cell proliferations showed positive c-MET staining (Table 1). Ten of 26 sarcomatoid mesotheliomas were positive for c-MET, with 4 of 26 (15%) showing an H-score >3 (chi-square = 11.1, df = 3, $p = 0.011$) (Table 1, Fig. 2).

Using an H-score >3 as the cutoff, c-MET membrane staining alone had a sensitivity of 53% and a specificity of 100% in distinguishing between EMM and E-RMP, whereas the sensitivity and specificity in distinguishing between SMM and S-RMP was 15% and 100%, respectively.

To determine whether there were important differences between TMA and whole-section scoring, we selected 7 cases of epithelioid mesothelioma that were scored as moderate or strong on the TMA and stained whole sections. In 4 cases, the score was the same on the TMA and whole section; in the other 3, the score increased from moderate on the TMA to strong on the whole sections, largely reflecting more widespread staining visible on the whole sections.

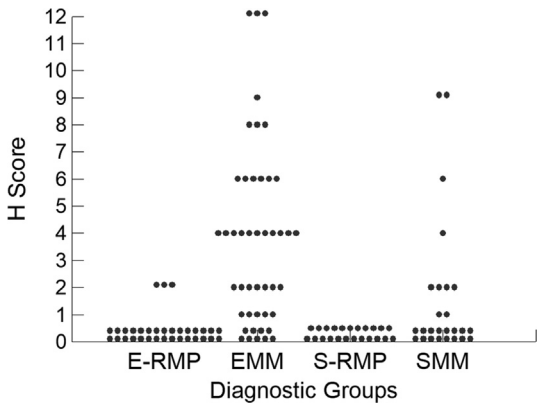

Fig. 1 H-scores by diagnostic groups. E-RMP, epithelial reactive mesothelial proliferation; EMM, epithelioid mesothelioma; S-RMP, spindle cell reactive mesothelial proliferation; SMM, sarcomatoid/desmoplastic mesothelioma.

Table 1 c-Met membranous staining scores from both TMAs combined.

Staining	Negative	Trace	Moderate	Strong
E-RMP	30	3	0	0
S-RMP	23	0	0	0
EMM	9	12	17	7
SMM	16	6	2	2

Abbreviations: E-RMP, epithelial reactive mesothelial proliferation; EMM, epithelioid mesothelioma; S-RMP, spindle cell reactive mesothelial proliferation; SMM, sarcomatoid/desmoplastic mesothelioma; TMA, tissue microarray.

3.2. HER3 staining

HER3 membrane staining was found in 8 of 44 (18%) (Fig. 2) evaluable cases of EMM and 1 of 26 (4%) SMMs. The scores for EMM ranged from 1 to 12 (median = 5), and for the solitary positive SMM, the score was 1. No E-RMP or S-SMP cases showed HER3 staining.

3.3. BAP1 and MTAP staining

Of the cases with c-MET staining, 42 of 45 EMMs, 24 of 26 SMMs, 32 of 33 E-RMPs, and 22 of 23 S-RMPs were interpretable for BAP1 (Table 2) and 43 of 45 EMMs, 24 of 26 SMMs, 33 of 33 E-RMPs, and 22 of 23 S-RMPs were interpretable for MTAP (Table 3).

For EMM, BAP1 had an overall sensitivity and specificity of 50% and 100%, respectively, and MTAP had an overall sensitivity and specificity of 37% and 100%, respectively. Of the EMM cases with negative or trace membranous c-MET staining (H-score ≤ 3), BAP1 was lost in 8 cases and MTAP was lost in 9 cases. When c-MET scores ≤3 and BAP1 loss were used in conjunction, the sensitivity was 76%. When c-MET scores ≤3 and MTAP loss were used in conjunction, the sensitivity was 77%.

For SMM, BAP1 had a sensitivity and specificity of 21% and 100%, respectively, and MTAP had a sensitivity and specificity of 63% and 100%, respectively. Of the SMM cases with negative or trace (H-score ≤ 3) c-MET staining, BAP1 was lost in 5 cases and MTAP was lost in 13 cases. When c-MET and BAP1 were used in conjunction, the sensitivity was 38%. When c-MET and MTAP were used in conjunction, the sensitivity was 71%.

The combination of MTAP and BAP1 staining alone showed loss of one or both markers in 30 of 45 (67%) EMMs and 13 of 26 (50%) SMMs. The combination of c-MET staining >3 and/or BAP1 and/or MTAP loss was found in 36 of 42 (86%) EMMs and 15 of 22 (68%) SMMs.

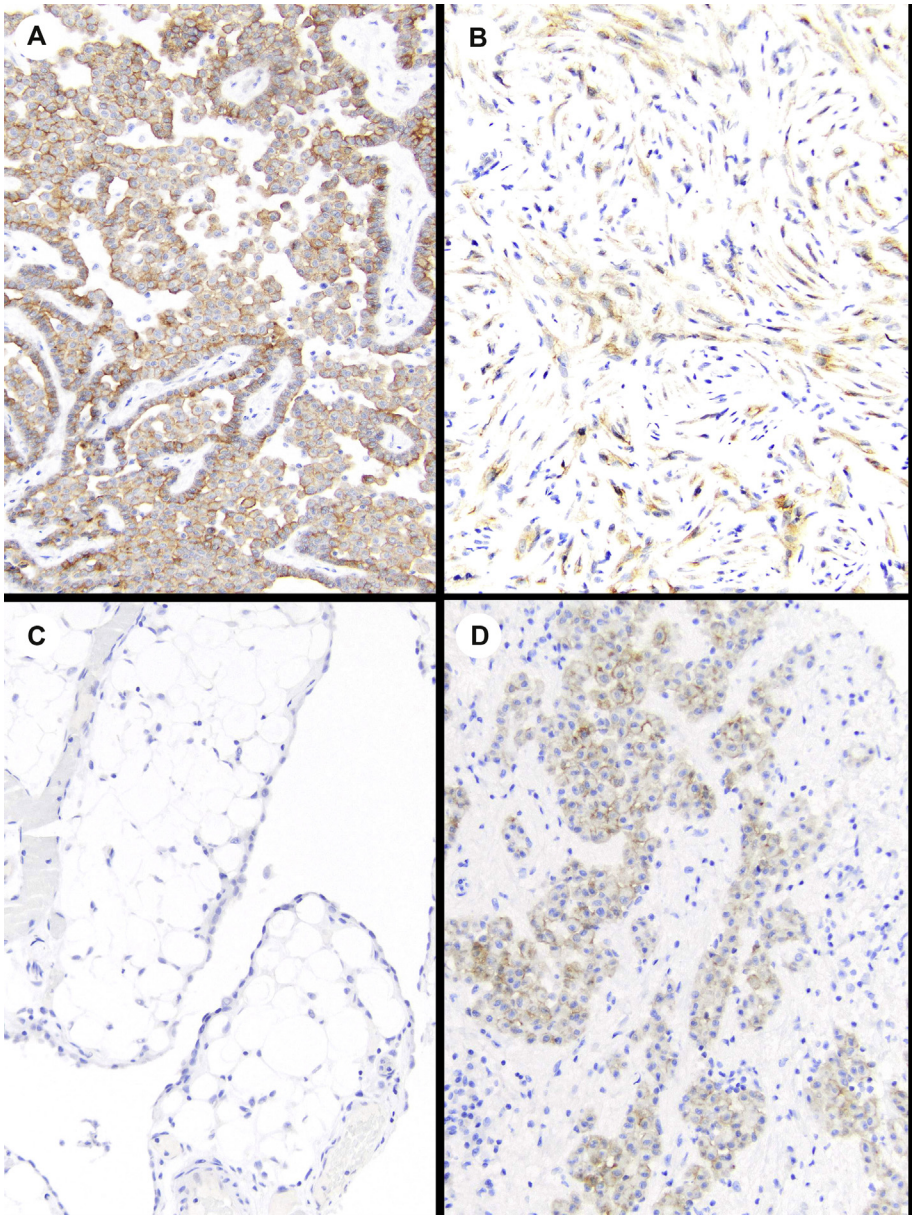

Fig. 2 Examples of immunohistochemical results. A, An epithelioid mesothelioma case stained for c-MET. B, A sarcomatoid mesothelioma case stained for c-MET. C, A completely negative reactive epithelial proliferation case (c-MET staining). D, HER3 staining in an epithelioid mesothelioma case. HER3, human epidermal growth factor receptor-3.

4. Discussion

MET pathway activation may be driven by a variety of mechanisms, including *MET* amplification, mutation, or translocation, leading to increased c-MET protein production or increased production of HGF, the ligand for c-MET [7,12]. HGF is normally produced in mesenchymal cells but can be aberrantly expressed in mesotheliomas and other malignancies, and production of both HGF and c-MET in the same cell leads to autocrine activation of the c-MET receptor [13]. Like other tyrosine kinases, mutated c-MET protein may also be constitutively active in the absence of the ligand [13].

The exact mechanism behind increased c-MET protein expression in malignant mesotheliomas is unclear, but immunohistochemically, detectable c-MET protein is quite common in these tumors. Bois et al. [8] found that c-MET staining was present in 147 of 149 mesotheliomas; of note, only 1 of their cases showed *MET* amplification by FISH, and only 1 case showed duplication of chromosome 7, the location of *MET*. Levallet et al. [7] observed c-MET membranous staining in 119 of 157 (76%) mesotheliomas.

Table 2 Comparison of c-MET and BAP1 staining.

c-MET staining	Negative	Trace	Moderate	Strong
EMM				
BAP1 retained	4	6	9	2
BAP1 loss	3	5	8	5
Not interpretable	2	1	0	0
SMM				
BAP1 retained	10	5	2	2
BAP1 loss	4	1	0	0
Not interpretable	2	0	0	0

Abbreviations: EMM, epithelioid mesothelioma; SMM, sarcomatoid/desmoplastic mesothelioma.

Table 3 Comparison of c-MET and MTAP staining.

c-MET staining	Negative	Trace	Moderate	Strong
EMM				
MTAP retained	5	5	11	6
MTAP loss	3	6	6	1
Not interpretable	1	1	0	0
SMM				
MTAP retained	5	2	1	1
MTAP loss	9	4	1	1
Not interpretable	2	0	0	0

Abbreviations: EMM, epithelioid mesothelioma; SMM, sarcomatoid/desmoplastic mesothelioma; MTAP, methylthioadenosine phosphorylase.

Thirkettle et al. [10] reported a positive result in 29 of 29 cases with both cytoplasmic and membrane staining. Zimmerman and Fogt [14] reported cytoplasmic but not membrane staining in 38 of 42 (90%) malignant mesotheliomas in effusion specimens. Jagadeeswaran et al. [2], using a polyclonal antibody, found that 54 of 66 (82%) of mesothelioma cases were positive, and their illustrations show both membranous and cytoplasmic staining. Harvey et al. [9] described some cases with cytoplasmic staining and some cases with both cytoplasmic and membranous staining using a polyclonal antibody on a small series (9 cases).

Bois et al. [8] also noted that epithelioid mesotheliomas tended to express c-MET more intensely than sarcomatoid mesotheliomas. Levallet et al. [7] similarly found that c-MET staining is more frequent in epithelioid mesotheliomas (87% of cases in their series) than in sarcomatoid mesotheliomas. Our data are similar: only 4 of 26 (15%) sarcomatoid mesotheliomas showed greater than trace staining, compared to 24 of 45 (53%) epithelioid mesotheliomas.

There is very little information in the literature on staining of benign mesothelial cells for c-MET, and the extant data are contradictory. Jagadeeswaran et al. [2] found that none of 20 normal mesothelial samples were c-MET positive. In contrast, Thirkettle et al. [10] reported that 4 of 4 samples of reactive pleura were positive, although the staining was purely cytoplasmic, and the authors also used a polyclonal antibody. Zimmerman and Fogt [14] observed positive staining in 18 of 34 (53%) benign reactions; again, they used a polyclonal antibody. In contrast, in more recent reports using monoclonal antibodies, c-MET staining in mesothelioma may be membranous or membranous and cytoplasmic [7,8], an observation confirmed here, but apparently never cytoplasmic alone. Our data suggest that c-MET staining is relatively uncommon in reactive mesothelial proliferations; none of the 23 reactive spindle cell proliferations was c-MET positive, and only 3 of 33 reactive epithelial proliferations showed any c-MET staining, all of the latter scored as trace (H-score ≤ 3).

In normal cells, HER3 is phosphorylated by epidermal growth factor receptor (EGFR) or HER2, and HER3 signaling enhances cell survival. In non-neoplastic cells, c-MET does not phosphorylate other receptor tyrosine kinases, but in neoplastic cells, overexpressed c-MET can phosphorylate HER3, and this process is believed to be a mechanism of drug resistance [6]. Given this mechanism, we examined staining for HER3 as a potential marker of malignancy, but the results were disappointing, with only a very small percentage of positive tumors (Fig. 2D), all but one of which was also positive for c-MET. For this reason, we did not investigate staining for phosphorylated HER3, which in this context would be a more specific marker of c-MET activity.

Like all of the reported markers for separating benign from malignant mesothelial proliferations, c-MET staining needs to be applied to the appropriate morphologic pattern (epithelial vs spindle cell), and a staining cutoff and/or intensity needs to be determined; the latter is a recurring problem for most of the published markers in this area (reviewed in the study by Churg and Naso [1]). Our data indicate that c-MET staining can be used to support a diagnosis of epithelioid mesothelioma, and improved sensitivity can be achieved by using a combination of c-MET and BAP1 or c-MET and MTAP staining. Application of H-scores as described here is not a practical proposition for most laboratories unless they see many mesotheliomas, and we suggest that easily visible moderate or strong membrane staining (Fig. 2A or c-MET staining similar to that shown for HER3 in Fig. 2D) can serve instead as an indicator of malignancy. For potential sarcomatoid mesotheliomas, sensitivity of c-MET staining is too low to make this a worthwhile test.

Author contributions

H.Z.R. contributed to study design, data acquisition, data analysis, and manuscript writing. S.C. contributed to

data acquisition. A.C. contributed to study design, data acquisition, data analysis, and manuscript writing.

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