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Prognostic Value of c-MET Expression in Patients With Pancreatic Cancer Receiving Adjuvant and Neoadjuvant Chemoradiation Therapy

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Abstract

Purpose: To determine the prognostic significance of c-MET expression and develop a predictor of distant failure in patients with resectable pancreatic cancer treated with chemoradiation.

Methods and Materials: We used a tissue microarray to study protein expression by immunohistochemistry in 102 patients treated surgically for pancreatic cancer. Two cores per patient were blindly scored from 0 (no staining) to 3 (strong staining) by a single pathologist. The Kaplan-Meier method was used to determine time to local and distant failure, overall survival, and progression-free survival. *P* values were calculated with the log-rank test.

Results: High tumor expression of c-MET was associated with a shorter time to distant failure in patients receiving neoadjuvant (n=23) or neoadjuvant therapy (n=73) (median 8.9 months vs 22.0 months, *P*=.0010). We then examined the ability of incorporating 2 known biomarkers, thymidylate synthase and DPC4 (SMAD4), with c-MET to risk-stratify patients. This multi-protein predictor divided our cohort into groups of similar numbers and was predictive of distant failure (median 13.4 months vs 24.2 months, *P*=.0094) but not of local control.

Conclusion: c-MET is potentially predictive of distant failure. Using c-MET, DPC4, and thymidylate synthase, we developed a multi-protein predictor that could be used to risk-stratify patients and guide decisions regarding the sequencing of locoregional and systemic therapies in pancreatic cancer.

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Introduction

The benefit of adjuvant chemoradiation therapy in patients with resected pancreatic cancer has not been confirmed in a modern phase 3 trial and has been questioned in recent studies (1). Radiation therapy improves local control; however, given the high metastatic potential of this disease, the benefit of local control is diminished in a nonselected population. Still, local failure leads to substantial morbidity: approximately one-third of patients with pancreatic cancer succumb as a result of complications from uncontrolled local disease (2, 3). As more effective systemic therapies are developed, the impact of local control will increase. Alternatively, more aggressive locoregional therapies could benefit the subset of patients who are less likely to develop metastases. Biomarkers predictive of distant failure have the potential to individually tailor adjuvant therapy.

Multiple prognostic biomarkers have been studied in pancreatic cancer, including DPC4 and thymidylate synthase (TS). Previous studies have shown an association between loss of DPC4 and a metastatic phenotype. A rapid autopsy study has demonstrated that pancreatic cancers with loss of DPC4 are associated with widespread metastatic disease (3). Additionally, evaluations of surgical specimens reveal that in patients with resectable pancreatic cancer, the loss of DPC4 is associated with distant failure (4).

Thymidylate synthase is the ultimate enzymatic target of 5-fluorouracil (5-FU) and has been identified as a predictive biomarker in patients receiving 5-FU chemotherapy. It has been most thoroughly studied as a biomarker of response in colorectal cancer, where high expression by immunohistochemistry (IHC) is associated with a lesser response to therapy (5, 6). Additionally, TS expression is prognostic in breast, esophageal, and gastric cancer (7–9). In pancreatic cancer, a study by Hu et al (10) found that high TS expression was associated with poorer survival in patients who underwent surgery. In contrast, smaller studies examining the prognostic utility of TS in pancreatic cancer have demonstrated conflicting results (11).

c-MET is a receptor tyrosine kinase that regulates multiple cancer-related processes. In several disease sites, high expression of c-MET is associated with a poor prognosis and a distant failure phenotype (12). c-MET activation induces an invasive growth program, characterized by cell spreading, cell–cell dissociation, motile phenotype acquisition, migration, and proliferation (13–15). In consideration of these reports, we hypothesized that c-MET is associated with distant failure in patients with pancreatic cancer.

In the present study we used a tissue microarray (TMA) to examine protein expression of potential biomarkers of distant metastatic disease in patients with resected pancreatic cancer who had received neoadjuvant or subsequently underwent adjuvant chemoradiation therapy. Our first aim was to determine whether c-MET was associated with time to distant failure in patients receiving neoadjuvant or adjuvant chemoradiation. We then used the TMA to study a combination of markers to optimize our ability to distinguish patients according to their risk of distant failure.

Methods and Materials

Tissue microarray

Following institutional review board approval, primary tumor samples from patients with pancreatic cancer who underwent resection between June 2002 and August 2008 were used to create three TMAs. One of the TMAs included specimens from patients treated with neoadjuvant chemoradiation therapy, and the other 2 TMAs included patients who had no treatment before surgery.

Two cores, previously fixed and embedded in paraffin, were obtained from each resection. Sections of the TMA were evaluated for each protein marker by IHC, with all cores stained at the same time and analyzed together. The specimens were between 3 and 9 years old when the TMA was created. The TMA was stained and analyzed approximately 1 year after its creation. Control tissues were also included on the TMA for confirmation of staining. Protein expression was scored as 0 (no staining), 1 (mild), 2 (moderate), or 3 (strong) by a single pathologist blinded to the subjects' treatment and outcome.

For c-MET, cytoplasmic (vs nuclear) staining was scored. The two cores were averaged, and the patients were dichotomized according to our initial hypotheses that high c-MET expression (IHC score 2–3) would be associated with decreased time to distant progression. On the basis of prior studies, for DPC4 (SMAD4) we looked for a group that had evidence of DPC4 loss, defined in at least 1 core with an IHC score of 0. For TS, nuclear staining was used, which has been shown to be predictive in prior reports (10).

Endpoints

For time to distant failure, an event was defined as radiographic or clinical progression or the finding of metastatic disease at the time of surgery. Patients were censored at last follow-up or at time of death if the patient died without evidence of disease. For progression-free survival and overall survival, patients were censored at the time of last follow up if no event had occurred. All endpoints were calculated from the date of diagnosis.

Statistical analysis

The product-limit method of Kaplan-Meier was used to calculate overall survival, progression-free survival, and time to distant and local progression. The log-rank test was used to compare groups. To confirm our findings, we also calculated these endpoints in patients who received neoadjuvant therapy separately from patients who had initial surgery, given the potential effect of chemoradiation on tumor marker staining.

Results

Patient characteristics and treatment

There were 77 patients in the adjuvant therapy cohort and 25 patients in the neoadjuvant therapy cohort. All of the patients treated with neoadjuvant therapy received gemcitabine-based chemoradiation; 18 of these patients also received oxaliplatin. Neoadjuvant radiation targeted the tumor and involved lymph nodes. No elective nodal radiation therapy was

performed. In patients who had surgery up front, 54 patients received postoperative chemoradiation therapy with either capecitabine or 5-FU, 1 patient received radiation with concurrent gemcitabine, and 22 patients did not receive chemoradiation therapy. Adjuvant chemoradiation therapy targeted the tumor bed and at-risk lymphatics. The distributions of age and performance status for all patients are displayed in Table 1. Most of the patients had a Zubrod performance status of 0 or 1 (84%). Sixty-six of the 102 patients received chemotherapy (distinct from chemotherapy concurrent with radiation therapy). The most commonly used chemotherapy drug was gemcitabine, which 84 patients received, often in combination with cisplatin (n=37), oxaliplatin (n=20), or capecitabine/5-FU (n=17).

Tumor staining

Two tumor cores were obtained and stained for each subject. The 2 IHC scores for each tumor were averaged. There were 2 patients who had no detectable tumor for both of their core specimens. These patients were excluded from the analysis. For patients for whom only 1 core contained enough cancer cells to score, the single specimen staining score was used for the analysis (n=17 for DPC4, n=21 for TS, n=20 for c-MET). Table 2 shows the distribution of c-MET cytoplasmic staining. Interestingly, a similar distribution of high c-MET staining (IHC score of 2) was seen in patients receiving neoadjuvant therapy (20%) and patients who had surgery up front (21%).

In addition to c-MET, we also stained for DPC4 (SMAD4) and TS. For TS the average of the 2 cores was used, and a value of 1 was considered low. In the neoadjuvant group 40% had low staining, and in the adjuvant therapy group 61% of patients had low staining. For DPC4 we used the minimum value because loss of this protein (IHC score 0) is known to be prognostic, and given tumor heterogeneity it is possible for a portion of the tumor to have DPC4 loss and for another portion to have DPC4 intact. Distribution of no DPC staining was 20% in the neoadjuvant group and 13% in patients who had upfront surgery.

High cytoplasmic c-MET levels are associated with decreased time to distant failure and progression free survival

The product-limit method was used to determine time to progression in patient tumors with high and low c-MET staining. In all patients who underwent surgical resection, high levels of c-MET were associated with a shorter time to distant progression, with a median time of 8.9 months (95% confidence interval [CI] 6.0–20.3 months) compared with 22.0 months (95% CI 15.0–35.3 months) in patients with low c-MET staining ($P=.0010$; Fig. 1A). High c-MET staining was also associated with short progression-free survival (7.7 months vs 17.3 months; $P=.0037$; Fig. 1B). To internally validate these findings, we examined c-MET in patients who received neoadjuvant therapy separately from patients who received adjuvant therapy. High c-MET staining was significantly associated with a shorter time to failure in both cohorts (Fig. E1; available online at www.redjournal.org).

Predictors of distant metastasis

We next examined DPC4 and TS expression in our specimens, given their known predictive value in pancreatic cancer. Loss of DPC4 has been previously shown to be associated with a metastatic phenotype (3, 4). In our TMA, tumors with no DPC4 staining in at least 1 core

had a significantly shorter time to distant failure compared with patients who had DPC4 in their tumors (median in months: 9.0 [95% CI 4.0–20.3] vs 22.3 [95% CI 14.7–35.3]; $P=.016$; Fig. 2A). High nuclear TS was also associated with a shorter time to metastasis compared with tumors with low levels of nuclear TS (median in months: 10.7 [95% CI 6.9–22.0] vs 20.9 [95% CI 14.2–34.5]; $P=.045$; Fig. 2B).

Development of a multi-protein predictor of distant metastasis

We then evaluated the potential of using c-MET, DPC4, and TS together to identify patients at high risk of distant failure. For this analysis, patients who did not have complete data from tumor staining of all 3 markers were excluded unless they had a high-risk feature. High-risk features included high c-MET staining (IHC score ≥ 2), loss of DPC4 (IHC score of 0 in at least 1 core), or high nuclear TS staining (IHC score > 1). Eighty-one patients had complete staining data for all 3 markers or a high-risk feature. The multi-protein predictor of distant metastasis split our patient population into approximately equal cohorts: 35 without and 46 patients with at least 1 high-risk feature. In the high-risk cohort median time to distant failure was 13.4 months (95% CI 8.8–21.8 months), compared with 24.2 months (95% CI 14.2–39.6 months) in the low-risk cohort ($P=.0094$; Fig. 3A). Interestingly, time to local failure was not significantly different between the high- and low-risk groups ($P=.28$; Fig. 3B). Patients with a high-risk feature also had shorter overall survival (median 18.6 months vs 28.7 months; $P=.041$; Fig. 4A) and shorter progression-free survival (median 12.3 months vs 17.3 months; $P=.013$; Fig. 4B).

As a sensitivity analysis to missing marker staining for the 13 patients without complete marker data or at least 1 high-risk feature, those cases were considered to all be negative for each high-risk feature or alternatively positive for at least 1, in 2 separate analyses. Time to distant failure was again compared. In both analyses, cases with the high-risk feature had shorter median times free of distant failure than cases without (13.4 months [95% CI 7.6–21.8] vs 43.0 months [95% CI 17.3–89.6], $P<.001$; and 19.0 months [95% CI 9.0–23.6] vs 26.8 months [95% CI 13.1–89.6], $P=.058$, respectively) in the adjuvant cohort.

Discussion

In this study we identified c-MET as a predictor of distant failure and progression-free survival in patients with resectable pancreatic cancer. We demonstrated the predictive value of this protein in 2 separate patient cohorts who received different therapies. Additionally, we incorporated 2 known biomarkers for pancreatic cancer, DPC4 (3, 4) and TS (10, 11), to create a multi-protein predictor of distant metastasis in patients receiving multimodality therapy. The predictor divided our patient population in approximately half. In the high-risk group almost all patients developed metastasis within 3 years, and in the low-risk group almost half of the patients were without distant failure. Interestingly, the risk of local failure was similar in both groups.

Incorporating c-MET into our predictive model classified several patients with intact DPC4 and low TS as high risk. There were 10 patients who had c-MET as their only risk factor, compared to 9 patients with DPC4 and 16 patients with TS as their only risk factor. Overall,

35 of the 46 high-risk patients only had 1 high-risk feature, suggesting that each of these markers adds value to in predicting distant failure.

c-MET regulates several signal transduction pathways. The main ligand for c-MET is hepatocyte growth factor (HGF). Upon HGF binding, c-MET forms an active dimer that promotes signal transduction directly through its kinase activity and indirectly through the scaffolding protein Gab1 (16). c-MET can be activated in cancer through several mechanisms, the most common being overexpression at the transcriptional level. Furthermore, c-MET is up-regulated by hypoxia, promoter stimulation by HGF, and the downregulation of specific micro-RNAs. c-MET plays an important role in cellular processes associated with metastasis, including invasion and migration (15). It is not surprising, therefore, that high expression of c-MET is associated with distant failure.

c-MET plays an important role in pancreatic cancer. Both c-MET and HGF are overexpressed in pancreatic tumors compared with normal pancreatic tissue (17). A prior study examining 50 tumors has suggested that high c-MET expression in pancreatic cancer cells and high HGF expression in pancreatic tumor stroma are associated with disease-free survival (18). Another report found high levels of MET promoter hypomethylation in pancreatic cancer, and gene expression of MET correlated with overall survival in a 100-patient cohort (19). In the present study we demonstrate that high c-MET staining by IHC in tumors is associated with decreased time to metastasis and progression in 2 cohorts of patients receiving different therapies. Our study represents the largest analysis of c-MET as a prognostic marker of distant failure.

In this study we examined tumors that received neoadjuvant chemoradiation in addition to tumors that were resected before any treatment. A novel finding from this study is that the distribution of c-MET staining was similar in patients receiving neoadjuvant chemoradiation as compared with those patients who had surgery first. With the other markers examined, we also saw similar staining patterns between the 2 groups. Radiation therapy has been shown to induce c-MET expression in preclinical models (20). The similar staining pattern in patients receiving neoadjuvant and adjuvant therapy could be related to the long period between radiation and surgery (typically several weeks) and the transient nature of radiation protein expression. Alternatively, because local control rates were similar in patients who had high and low c-MET expression, this protein may be a marker of a metastatic phenotype, with no correlation to radioresistance.

The distribution of DPC4 staining was different in our study than what has been previously reported. In the study by Tascilar et al (4), 43% of tumors were classified as DPC4 negative. On the genetic level, approximately 35% of pancreatic cancers have homologous loss of SMAD4/DPC4, and another 20% have loss of 1 allele in addition to a mutation (21, 22). In our study only 13%–20% of samples had DPC4 loss by IHC. We can explain this discrepancy on the basis of the following factors. First, although IHC staining shows a high level of concordance with genetic loss of DPC4, approximately 10% of samples with genetic loss have positive staining by IHC (23). Prior studies also have used different cutoffs for negative staining. For example, in the report by Tascilar et al (4), up to 5% of cells could have positive staining for the sample to be called negative. Second, improved axial imaging

techniques likely selected out more patients with small-volume metastatic disease in our series than in previous reports from earlier eras. Interestingly, in patients with locally advanced pancreatic cancer who have no metastatic disease at the time of death, only 22% show DPC4 loss (3), which is similar to the distribution seen in our series. These findings suggest that the incidence of DPC4 loss in resectable patients may be considerably less in the current era, given stage migration.

A predictor of distant metastasis potentially has high clinical utility in pancreatic cancer. Local control with surgery provides patients the best chance of cure. In patients who are not surgical candidates the role of local therapy has been questioned in recent studies. In the LAP07 study, the use of radiation reduced local failure from 65% to 34% (24). This dramatic improvement was not associated with a survival benefit, given the high rate of distant failure in the study population. In patients with a lower risk of distant failure, an improvement in local control would likely be associated with an improvement in survival, as seen in other diseases such as breast and rectal cancer (25, 26). Future trials could potentially use this marker to risk-stratify patients after surgery. Patients at a low risk of distant failure would potentially benefit more from local control by receiving chemoradiation early in their treatment course. Patients with a high risk of distant failure would be better served by systemic therapy up front, with radiation reserved for those who do not develop distant metastases after a period of time.

The major limitation of the present study is a lack of an independent validation set. To overcome this issue we planned up front to analyze the neoadjuvant and adjuvant cohorts separately. After splitting the cohort, the number of patients in the neoadjuvant arm was small, but still a statistically significant difference was seen. Additionally, prior reports have demonstrated the prognostic potential of DPC4 (3, 4), TS (10), and c-MET (18) in pancreatic cancer. Missing data prevented classification of the multi-protein predictor for all samples; however, sensitivity analyses suggested the association with time to distant failure was still meaningful. Validation in a larger independent data set is needed to establish this predictor for clinical use.

In summary, in this report we describe the prognostic importance of c-MET expression and confirm the potential of c-MET to predict distant failure in patients with resectable pancreatic cancer. We demonstrate this effect in patients who received adjuvant and neoadjuvant therapy. Finally, using two established biomarkers, DPC4 and TS, in addition to c-MET, we developed a multi-protein predictor of distant failure that upon further validation could be used to risk-stratify patients and guide decisions regarding the sequencing of locoregional and systemic therapies in pancreatic cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Summary

A tissue microarray was used to study protein expression in 102 patients with pancreatic cancer. High tumor expression of c-MET was associated with a shorter time to distant failure in patients receiving neoadjuvant or adjuvant therapy. We then incorporated 2 known biomarkers, thymidylate synthase and DPC4, with c-MET to develop a predictor of distant metastasis. This multi-protein predictor divided our cohort into groups of similar numbers and was predictive of distant failure.

Fig. 1.

Association between c-MET expression and time to distant failure in patients with resectable pancreatic cancer. Immunohistochemistry (IHC) was performed on paired tumor samples obtained at the time of surgery. Each tumor was scored from 0 (no staining) to 3 (strong staining) for the cytoplasmic levels of c-MET, and the results were averaged. An average score of 2 was considered high c-MET staining. Subjects lost to follow-up or who had died without a known recurrence were censored. Shown are the Kaplan-Meier curves for time to distant failure (A). High c-MET expression was associated with a shorter time to distant failure in patients who received neoadjuvant or adjuvant therapy. Additionally, high tumor c-MET was associated with decreased progression-free survival (B) in all patients.

Fig. 2.

Predictors of distant failure in patients with resectable pancreatic cancer. Tumors from patients with resectable pancreatic cancer were obtained at the time of surgery. Immunohistochemistry (IHC) was performed for the proteins thymidylate synthase (TS) and DPC4 (SMAD4). A pathologist scored each stained section from 0 (no staining) to 3 (strong staining) from paired samples from each tumor. For TS a score greater than 1 was considered high risk, and for DPC4 the absence of staining in a core was considered high risk. Patients who did not experience a distant failure were censored at last follow-up or death. The loss of DPC4 (A) or high TS expression (B) was associated with a shorter time to distant failure in patients with resectable pancreatic cancer.

A Multi-Protein Predictor of Distant Failure After Surgery

B Multi-Protein Predictor and Local Progression

Fig. 3. Multi-protein predictor of distant failure. Immunohistochemistry was performed on tumor specimens obtained at the time of surgical resection for pancreatic cancer. Thymidylate synthase, DPC4 (SMAD4), and c-MET levels were scored for each paired specimen. Patients were considered high risk if they had a c-MET score ≥ 2 , a TS score >1 , or a DPC4 score <1 . If no high-risk features were present, the subject was considered low risk. Patients without tumor staining for each marker were excluded unless they had a high-risk feature. Shown are Kaplan-Meier curves for freedom from distant failure (A) and freedom from local failure (B). The multi-protein predictor divided the cohort into approximately equal parts and was predictive of distant but not local failure.

A Multi-Protein Predictor and Overall Survival **B Multi-Protein Predictor and Progression Free Survival** **Fig. 4.**

Multi-protein predictor and overall and progression-free survival. We examined the ability of the multi-protein predictor described in Fig. 3 to predict overall survival and progression-free survival. Patients with a high risk of distant failure also had shorter overall survival (A) and progression-free survival (B).

Table 1

Patient and treatment characteristics

Characteristic	n (%)
Age at diagnosis (y)	
<60	28 (27)
60–70	42 (41)
>70	32 (31)
Zubrod performance status at diagnosis	
0	50 (49)
1	36 (35)
2	3 (3)
Not reported	13 (13)
Chemoradiation regimen	
Adjuvant capecitabine with 45–54 Gy	50 (49)
Adjuvant 5-fluorouracil with 50–52 Gy	4 (4)
Neoadjuvant gemcitabine and oxaliplatin with 27–31.5 Gy	18 (18)
Neoadjuvant gemcitabine with 36 Gy	7 (7)
Adjuvant gemcitabine with 50 Gy	1 (1)
No chemoradiation	22 (22)
Pathologic T stage	
T1	8 (8)
T2	11 (11)
T3	83 (81)
Pathologic N stage	
N0	44 (43)
N1	56 (55)
Nx	2 (2)

Table 2

Immunohistochemistry staining summary

Parameter	Neoadjuvant therapy (n=25)	No prior treatment (n=77)
c-MET average IHC score		
0	5	10
0.5–1.5	13	47
2–3	5	16
Not assessable	2	4
TS (nucleus) average IHC score		
0–1	10	42
1.5–2	4	20
2.5–3	0	2
Not assessable	11	13
DPC4 (cytoplasm) minimum IHC score		
0	5	10
1	16	49
2–3	1	10
Not assessable	3	8

Abbreviations: IHC = immunohistochemistry; TS = thymidylate synthase.

The proportion of patients with a high-risk factor was not significantly different between the neoadjuvant and adjuvant groups (*t* test *P* values: c-MET, *P*=.37; TS, *P*=.68; DPC4, *P*=.99).