Clinical Cancer Research

Patient-Derived Xenograft Establishment from Human Malignant Pleural Mesothelioma

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Abstract

Purpose: Malignant pleural mesothelioma (MPM) is a rare but aggressive disease with few therapeutic options. The tumor–stromal interface is important in MPM, but this is lost in cell lines, the main model used for preclinical studies. We sought to characterize MPM patient-derived xenografts (PDX) to determine their suitability as preclinical models and whether tumors that engraft reflect a more aggressive biological phenotype.

Experimental Design: Fresh tumors were harvested from extrapleural pneumonectomy, decortication, or biopsy samples of 50 MPM patients and implanted subcutaneously into immunodeficient mice and serially passaged for up to five generations. We correlated selected mesothelioma biomarkers between PDX and patient tumors, and PDX establishment with the clinical pathologic features of the patients, including their survival. DNA of nine PDXs was profiled using the OncoScan FFPE Express platform. Ten PDXs were treated with cisplatin and pemetrexed.

Results: A PDX was formed in 20 of 50 (40%) tumors implanted. Histologically, PDX models closely resembled the parent tumor. PDX models formed despite preoperative chemotherapy and radiotherapy. In multivariable analysis, patients whose tumors formed a PDX had significantly poorer survival when the model was adjusted for preoperative treatment (HR, 2.46; 95% confidence interval, 1.1–5.52; $P = 0.028$). Among 10 models treated with cisplatin, seven demonstrated growth inhibition. Genomic abnormalities seen in nine PDX models were similar to that previously reported.

Conclusions: Patients whose tumors form PDX models have poorer clinical outcomes. MPM PDX tumors closely resemble the genotype and phenotype of parent tumors, making them valuable models for preclinical studies. *Clin Cancer Res; 23(4); 1060–7. ©2016 AACR.*

Introduction

Malignant pleural mesothelioma (MPM) is a rare but highly aggressive malignancy originating from the mesothelial cells. Until 50 years ago, it was a rare diagnosis; however, its increasing incidence has paralleled the use of asbestos in the postwar industrial period (1). Given its location, curative resection is often unable to be performed, and recent data suggest that this modality does not improve overall survival (2).

Although histologic classification has consistently shown the epithelioid subtype confers an improved prognosis compared with sarcomatoid or biphasic, other prognostic markers, such as neutrophil-to-lymphocyte ratio, and genomic alterations have been less clear (3–6). Overall, the prognosis of MPM patients remains poor and therapeutic options are limited. Recently studies adding bevacizumab to chemotherapy (7) and the use of immunotherapies have renewed enthusiasm in mesothelioma treatments, where treatment paradigms have remained unaltered since 2003 when the phase III EMPHACIS study was published (8). As compared with other tumor types, the addition of these active agents has also prompted a search for predictive biomarkers.

Most biomarker-based studies have focused on archival tissues and drawn correlations using retrospectively captured data. To determine functional biology, established cultured cell lines have traditionally been used as the major preclinical models to study human cancers, including mesothelioma. There are currently less than 20 commercially available cell lines to study human MPM, although ongoing tissue banking by various groups is likely to increase this number (9). More recently, patient-derived xenografts (PDX) in immunodeficient mice have been employed as tumor models, as they may better parallel the original tumor and subsequent data may be more clinically relevant. We have previously reported that PDX models of non–small cell lung cancer closely resemble the primary tumor they were derived from; however, tumors that engrafted into immunodeficient mice also represented an independently poorer phenotype compared with tumors that did not engraft (10). In MPM, few studies have reported the clinical utility of PDX models. The largest study to

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Translational Relevance

Until recently, most preclinical studies in mesothelioma used cell lines or xenografts established from them. However, highly passaged cell lines do not faithfully recapitulate the original tumor they were derived from, and their use as preclinical models is therefore uncertain, which may in part explain the high failure rate for new therapies. Furthermore, there is a paucity of mesothelioma cell lines. We demonstrate that patient-derived xenografts (PDX) models can be efficiently established in immunocompromised mice and better represent the original tumor. We also demonstrate that tumors that form PDX models are also associated with poorer patient survival. This makes these models valuable to study the aggressive biology of malignant mesothelioma.

date looked at five models and found them to strongly correlate with the primary lesion (11).

In this study, we attempted to establish 50 MPM PDX models from patients treated with extrapleural pneumonectomy (EPP), decortication, or excisional biopsy. We report herein the clinical and pathologic factors associated with engraftment and the response patterns of the models to cisplatin and pemetrexed.

Materials and Methods

Patients and tissue samples

According to a study design approved by the Research Ethics Board of the University Health Network, consenting MPM patients, who underwent surgery at the University Health Network, Toronto, Canada from 2005 to 2010, were included in this study. Surgical tissue samples were obtained, and clinical data were retrieved.

Establishment of xenograft model in NOD/SCID mice

NOD/SCID mice were bred and provided by the animal facility of University Health Network. Female 6- to 8-week-old mice were used for PDX development. Fresh human MPM samples (P0 = passage zero) were obtained directly from the operating room, dissected carefully, and trimmed into small pieces (approximately 1 mm^3 cubes) under sterile conditions and implanted subcutaneously into both flanks of the mice.

PDX development was assessed by palpation of the site of implantation. Once a tumor grew to 15 mm in diameter or if the animal developed sickness or ulceration of the tumor, the mouse was euthanized and harvested fresh tumor fragments were reimplanted into 5 mice for passaging. Each model derived from the individual patient was passaged up to five generations (referred to as P1–P5). At each passage, tumor tissue were cryopreserved (90% of FBS and 10% of DMSO), snap-frozen in liquid nitrogen for future molecular profiling, and fixed in neutral-buffered formalin for histologic examination. Animals in which implanted tumors failed to grow at 8 months were euthanized and defined as non-PDX forming.

Pathology and biomarker evaluation

The histopathology of the primary P0 tumors and the xenograft models from all passages was evaluated to confirm the presence of MPM in all cases. Immunohistochemistry was performed by standard method using the following antibodies: calretinin (clone DC8, Thermo Fisher), WT-1 (NCL-L-WT1-562, Leica Biosystems), and BRCA2-associated protein (BAP-1, clone C-4, Santa Cruz Biotechnology). FISH assay to assess the $p16^{INK4A}$ was performed using the Vysis LSI CDKN2A Spectrum Orange/Vysis CEP9 Spectrum Green probes (Abbott Molecular), according to a previously reported method (12). These marker studies were conducted on tissue microarrays constructed from the primary and xenograft tumors.

DNA extraction

Morphologically well preserved areas of tumor-rich P2 xenografts with minimal murine inflammatory cells were obtained using a 1-mm core needle. In addition, a matched nontumor tissue control, in the form of adjacent nonneoplastic lung from the P0 surgical specimens, was obtained for each sample. Tissue was digested with proteinase K, and DNA was isolated using the standard phenol–chloroform protocol. The proportion of contaminant murine DNA to human DNA was quantified with quantitative PCR using mouse primers (forward, 5′-GAGGCAGGATTTCTCTGTGTAACA-3′; reverse, 5′-GAGTTTTGAAGCCAGCCTAGTCTAA-3) and human primers (forward, 5′-CCAGGATGGTCTCGGATCTC-3′; reverse, 5′-CCTGTAATCCCAGCACTTTTGG-3′). Only samples with minimal to no murine DNA were included for genomic analysis.

Copy number analysis

High-resolution copy number analysis of nine PDX models was performed using OncoScan FFPE Express 330K MIP platform (Affymetrix). Tumor and nontumor samples were processed at the Affymetrix laboratory, which was blinded to the clinicopathologic features of the tumor samples (13, 14).

Analysis of the OncoScan MIP data was carried out using Nexus Copy Number v.6.0.beta (BioDiscovery). All samples had a median absolute pair-wise difference value of more than 0.6. Within each sample, probes that demonstrated a call rate of <90% and relative SD of >30% were excluded from further analysis. Probes were then clustered using the SNP-FASST2 algorithm. The median of the $\log_2$ ratio of each segment was calculated and used for copy number estimation. A segment was considered to have normal copy number (CN), CN gain, high gain, loss, and big loss if its log ratio value was zero, >0.3, >0.6, $\leq$0.3, and $\leq$0.6, respectively. Any altered chromosomal region with at least 75% reported CN variations (CNV), and those present in the control nontumor samples, were reported separately and excluded from further analysis.

Response of xenograft tumors to current chemotherapy

PDX tumors from second or more passages were implanted subcutaneously into both flanks of NOD/SCID mice. The mice were treated with chemotherapy once xenograft tumors became palpable (approximately 50 mm^3). Tumor-bearing mice at a tumor average of 5-mm diameter were divided into three groups, no treatment, cisplatin (5 mg/kg, Hospira Healthcare Corporation), and pemetrexed (200 mg/kg, Eli Lilly), with 5 to 10 mice per group. Treatment was given once weekly for three doses through tail vein injection. Response to treatment was evaluated by assessing tumor volume; two maximal perpendicular diameters of the tumors were measured once weekly with a caliper. Tumor volume was calculated according to the formula $V = ab^2\pi/6$, where a and b represent the longest perpendicular diameters (15).

Statistical analysis

A linear mixed effects regression model was used to model the relationship between time to sacrifice of mice and passage number. To account for heterogeneity between patient models, patient was included as a random effect. The associations between the main clinical factors and the establishment of a PDX were tested using the Fisher exact test for the categorical variables and the Mann–Whitney test for the continuous variable (age). The effect of the PDX engraftment on survival, not adjusted and adjusted for clinical factors, was tested within a Cox proportional hazards model. As only 24 deaths were observed, no more than two covariates were included in any model at a time. The graphs were produced using the Kaplan–Meier method. To determine the response of each patient model to treatment with cisplatin or pemetrexed, a linear mixed effects model was used to estimate the growth rates for each patient model and treatment. To stabilize the variance, tumor volume was log transformed. A P value of less than 0.05 was considered significant.

Results

Patients included

Fifty MPM cases were included (Table 1). The median age of patients at diagnosis was 62 (range, 32–77) years. Forty (80%) of the patients were male and 10 (20%) were female. Most patients (37/50; 74%) had epithelioid histology, three (6%) sarcomatoid, and ten (20%) were biphasic tumors (Table 1).

Surgical treatment with EPP

Radical surgery was performed using standard techniques. Thirty four (68%) of the 50 patients received EPP with an *en bloc* resection of the lung, pleura, ipsilateral hemidiaphragm, and pericardium, whereas the remainder 16 (32%) underwent either decortication (9/16) or biopsy (7/16; Table 1). All patients were followed-up according to the clinical protocol established at the University Health Network.

Neoadjuvant chemotherapy and high-dose hemithoracic radiotherapy

Among the 50 patients, 32 (64%) received preoperative chemotherapy, which consisted of three cycles of pemetrexed 500 mg/m^2 or gemcitabine 1,000 mg/m^2 plus cisplatin 75 mg/m^2 (16, 17). Six (12%) patients were treated with preoperative radiation. Only 1 patient received both preoperative chemotherapy and radiation, and 6 (12%) patients were treated with adjuvant hemithoracic radiation to a total dose of 54 Gy after EPP treatment. There were 11 (22%) palliative patients who did not receive either induction chemotherapy or adjuvant radiotherapy (Table 1).

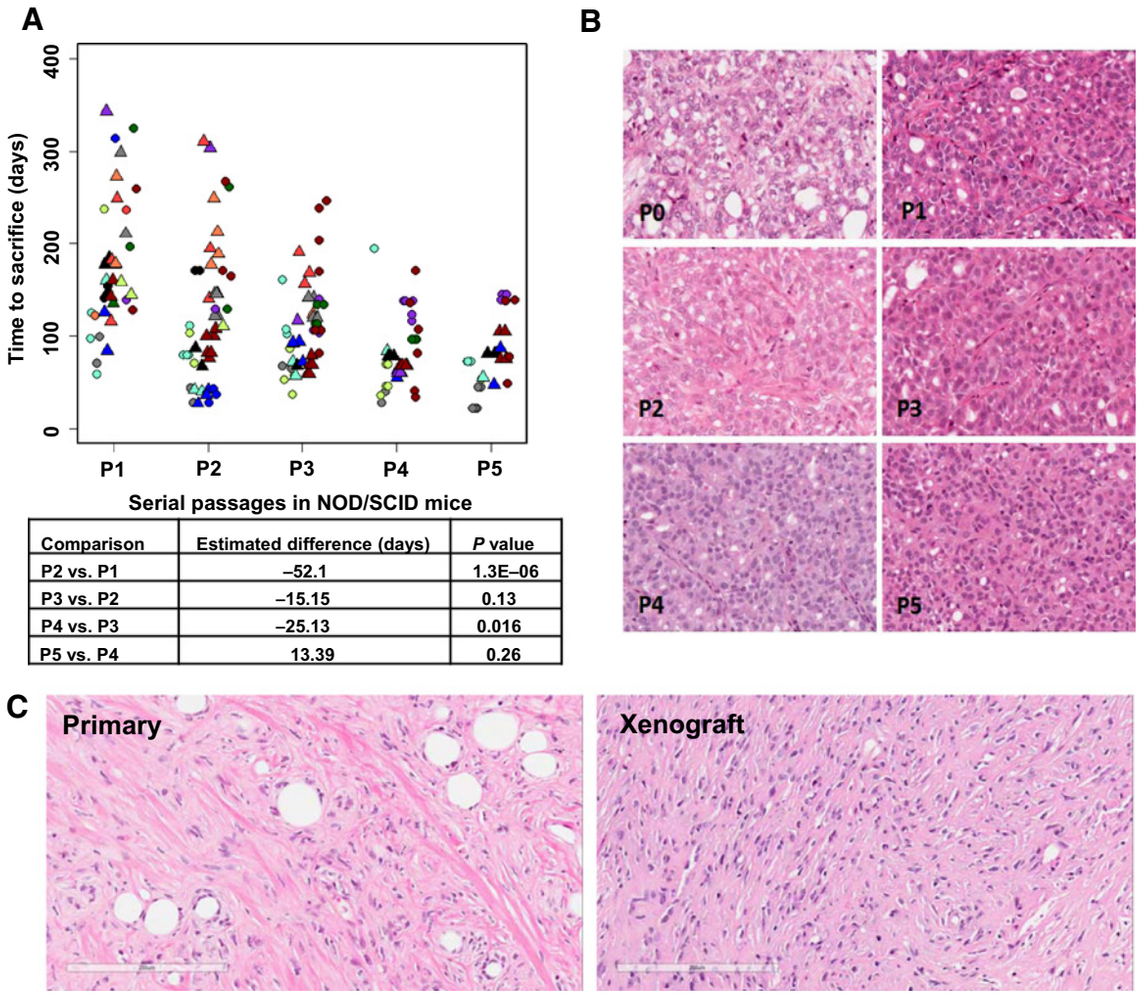
Development of MPM xenograft in NOD/SCID mice

Twenty PDX models were established from 50 MPM tissue implantations, an engraftment rate of 40% (20/50). The second-generation models required significantly less time to reach the humane endpoints compared with first-generation models (Fig. 1A, estimated difference −52 days, $P < 0.0001$). The time to endpoint decreased further in the third generation, but the difference between the third and second generation was not significant (Fig. 1A, estimated difference −15 days, $P = 0.13$). There was a significant difference in time to endpoint between the fourth and third passage (Fig. 1A, estimated difference −25 days, $P = 0.016$). However, the estimated time to humane endpoints did not differ significantly between the fifth and fourth passage (Fig. 1A, estimated difference = 13 days, $P = 0.26$). The engraftment rate for epithelioid tumor (12/37, 32%) was lower than that of other histologies (sarcomatoid, 2/3, 67%; biphasic, 6/10, 60%), but the difference was not statistically significant and limited by the low numbers of the

Table 1. Characteristics of the mesothelioma patients in this study

Attribute	Patient/tumor features	Total number	No PDX ($n = 30$)	PDX ($n = 20$)	P[a]
Age		n/a	62 (47–77)	62 (32–68)	0.67[b]
Gender	F	10	7 (70%)	3 (30%)	0.72
	M	40	23 (58%)	17 (42%)	
Pathologic T stage	T1/2	7	7 (100%)	0 (0%)	
	T3	11	8 (73%)	3 (27%)	0.056[d]
	T4	26	14 (54%)	12 (46%)	
	Tx[c]	6	1 (17%)	5 (83%)	
Pathologic type	Epithelioid	37	25 (68%)	12 (32%)	
	Sarcomatoid	3	1 (33%)	3 (6775%)	0.1[e]
	Biphasic	10	4 (40%)	6 (60%)	
Type of surgery	Biopsy	7	1 (14%)	6 (86%)	
	Decortication	9	6 (67%)	3 (33%)	0.13[f]
	EPP	34	23 (68%)	11 (32%)	
Preoperative treatment	No treatment	11	6 (55%)	5 (45%)	0.74
	Treatment	39	24 (62%)	15 (38%)	
Preoperative treatment	No treatment	11	6	5	
	Chemotherapy	32	19	13	
	Radiation	6	5	1	
	Combined	1	0	1	

Abbreviations: F, female; M, male.
[a]The P value is based on the Fisher exact test unless otherwise indicated.
[b]The P value is based on Mann–Whitney test.
[c]Nonsurgical advanced mesothelioma patients.
[d]P value is calculated for comparison between T1/2, T3, and T4.
[e]Comparison between epithelioid and the rest
[f]P value is calculated for EPP versus the rest.



Comparison	Estimated difference (days)	*P* value
P2 vs. P1	–52.1	1.3E–06
P3 vs. P2	–15.15	0.13
P4 vs. P3	–25.13	0.016
P5 vs. P4	13.39	0.26

Figure 1.

A, Time between tumor implantation and sacrifice of mice. Tumor tissues obtained from surgical specimens of patients with MPM were implanted subcutaneously into both flanks of NOD/SCID mice. Time between implantation and endpoints (tumor diameter reached 15 mm, or ulcerated tumor or mice are severely sick) are recorded for all serial passages. Ten cases have been passaged up to five generations. The estimated difference in time to sacrifice between generations was shown in the table. **B,** An example of serial passages of xenograft tumors in NOD/SCID mice from the patient MPM9. Histology of this case showed epithelioid subtype (H&E, 200×). **C,** A desmoplastic mesothelioma case that demonstrates a remarkable similarity in the histology of the primary (left) and xenograft (right) tumors.

sarcomatoid variant. For 34 patients who were treated by EPP, there was a similar rate of engraftment (11/34, 32%) as those treated by decortication (3/9, 33%). However, the engraftment rate for the biopsy patients was much higher (6/7, 86%). The rate of engraftment in the patients who received neoadjuvant chemotherapy was 42% (14/33), which was similar to untreated patients (45%, 5/11), but in those who were treated by hemithoracic high-dose radiotherapy, the engraftment rate was lower (29%, 2/7).

Genotypic and phenotypic comparison between patient and xenograft tumors

The histologies of the primary tumors were largely concordant to those of the xenograft tumors and were maintained with multiple passages, as exemplified by MPM-9 patient (Fig. 1B). All 12 epithelioid and two sarcomatoid mesotheliomas that formed xenografts showed identical histology in the latter. Among six biphasic tumors, two gave rise to xenografts with both sarcomatoid and epithelioid components, whereas four others gave rise to either epithelioid only ($n = 2$) or sarcomatoid only ($n = 2$) xenografts. Remarkably, two of the sarcomatoid mesotheliomas with desmoplastic histology showed similar features in their corresponding xenografts (Fig. 1C). By FISH, the $p16^{INK4A}$ copy number changes found in the patient tumors were also preserved in the corresponding xenografts (Table 2). Similarly, complete concordance of calretinin and WT-1 protein expression by IHC was also noted (Table 2; Supplementary Fig. S2). In a biphasic mesothelioma (MPM 18), calretinin staining was positive only in the epithelioid component but was negative in the sarcomatoid component. The xenograft grew only the sarcomatoid component, which was calretinin negative. BAP-1 nuclear staining by IHC was lost in 7 of 9 (78%) patient tumors

Table 2. Comparison of histology and selected biomarkers in primary tumors and xenografts in nine cases with matching samples available for analyses

		P16 by (FISH)			Calretinin (IHC)		WT-1 (IHC)		BAP1 (IHC)	
Sample ID	Primary histology	Patient tumor	PDX	PDX histology	Patient tumor	PDX	Patient tumor	PDX	Patient tumor	PDX
MPM 1	Biphasic	Homozygous deletion	Homozygous deletion	Biphasic	FP	FP	N	N	N	N
MPM 5	Biphasic	Hemizygous deletion	Hemizygous deletion	Epithelioid	P	P	P	P	N	N
MPM 7	Epithelioid	Homozygous deletion	Homozygous deletion	Epithelioid	P	P	N	N	N	N
MPM 9	Epithelioid	Homozygous deletion	Homozygous deletion	Epithelioid	P	P	P	P	N	N
MPM 17	Sarcomatoid	Homozygous deletion	Homozygous deletion	Sarcomatoid	N	N	N	N	P	P
MPM 18	Biphasic	Homozygous deletion	Homozygous deletion	Sarcomatoid	FP (E only)	N	N	N	N	P
MPM 24	Epithelioid	Homozygous deletion	Homozygous deletion	Epithelioid	P	P	P	P	N	N
MPM 32	Biphasic	2 copies and hemizygous deletion minor clone	2 copies and hemizygous deletion	Sarcomatoid	FP	FP	N	N	N	N
MPM 36	Epithelioid	Hemizygous deletion	Mixed homo/hemizygous deletion	Epithelioid	P	P	P	P	P	P

Abbreviations: E, epithelioid component; FP, focal positive; N, negative; P, positive.

studied. All xenografts showed concordant BAP-1 staining except in MPM18, with the primary tumor staining negative and the xenograft showing strong staining. The loss of BAP-1 nuclear staining has been reported in up to 70% of malignant mesothelioma and was highly correlated with the presence of BAP-1 mutation and/or gene copy loss (16–18).

Association between patient overall survival and xenograft development in mice

For all 50 MPM patients, the median follow-up time was 16.9 months (range, 3.7–79.2 months). The overall survival at 1, 2, and 3 years after diagnosis was 79%, 46%, and 33%, respectively (Fig. 2A). At the time of last follow-up, 24 patients had died (48%). The survival was not significantly different between epithelioid and biphasic/sarcomatoid patients in this cohort (Fig. 2B). On the basis of a Cox proportional hazards model, patients whose tumors did not engraft (non-PDX) had significantly better survival than those that did [PDX; $P = 0.028$, HR, 2.46; confidence interval (CI) of HR, 1.1–5.52; Fig. 2C]. Similar results were found in the epithelioid subset (HR, 3.2; 95% CI, 1.25–8.07; $P = 0.015$; Fig. 2D).

Adjusted effect of PDX development and survival

Three covariates were important to adjust the model. However, as there were only 24 events included in the model, only one other covariate could be included in addition to PDX development. In this way, we built three Cox models, each containing the xenograft covariate and one other adjustment covariate (Supplementary Table S1A–S1C). The analyses suggest that the effect of engraftment remained large (HR $\sim$ 2) regardless of which covariates were used in the model. The significance was preserved when the model was adjusted for preoperative treatment ($P = 0.026$, Supplementary Table S1C), but the P value for PDX formation was marginally nonsignificant after adjusting for either type of surgery ($P = 0.083$, Supplementary Table S1A) or histology ($P = 0.051$, Supplementary Table S1B). Although the association between PDX and the type of surgery was not significant (32% PDX in EPP group and 56% PDX in non-EPP group), it could have affected the significance of PDX formation in the Cox model ($P = 0.083$, Supplementary Table S1A). It is possible that the surgical mode was chosen based on the disease presentation characteristics, which could also have influenced the PDX success rate.

Copy number analysis

DNA was extracted from 16 P2 mesotheliomas. One sample had low-quality DNA, and three had high murine DNA content. Of the remaining 12 samples, nine P2 mesotheliomas were analyzed with OncoScan. The most common losses include losses in 1p, 3p, 6q, 9p, 10q, 13q, 14q, 15q, 16q, and 18q and gains in 1q, 3p, 5p, 6p, 7p, chr8, chr12, 15q, 16q, 17q, 20q, and 21q (Fig. 3). Most of these changes are similar to previously reported copy number changes in malignant mesotheliomas (19–23).

Response of PDX models to cisplatin and pemetrexed treatment

Ten PDX models were treated with cisplatin (Supplementary Table S2). In seven of these models, the tumor growth rate was considerably lower in mice treated with cisplatin than in control mice. These models were considered to have responded to cisplatin treatment (Fig. 4, Supplementary Fig. S1). If we based response to treatment on the difference in the growth rates between treatment and control mice, and selected 0.015 as the cutoff for what was considered a response, then three models were clearly nonresponders (MPM32, MPM49, and MPM36). Response to cisplatin occurred in five PDX models established from patients previously treated with neoadjuvant platinum-based chemotherapy; 4 of these 5 patients experienced disease control prior to surgery. Pemetrexed (Lilly Pharmaceuticals) was also used as a single agent and in combination with cisplatin. Single-agent pemetrexed did not demonstrate significance response (data not shown). The combination of cisplatin and pemetrexed did not result in any further responses above that seen with cisplatin alone (Supplementary Table S2).

Discussion

In an aggressive disease with limited therapeutic options, models of mesothelioma that enable functional studies with direct translatability into the clinic are an invaluable resource. Here, we have demonstrated that PDX models of MPM can be derived from all histologically subtypes and from small biopsy specimens. Importantly, both the morphology and characteristic genotypic and phenotypic markers of the primary lesion were mostly retained in the PDX. However, tumors that form PDXs are independently associated with poorer outcome regardless of histology or treatment when compared with tumors that did not engraft.

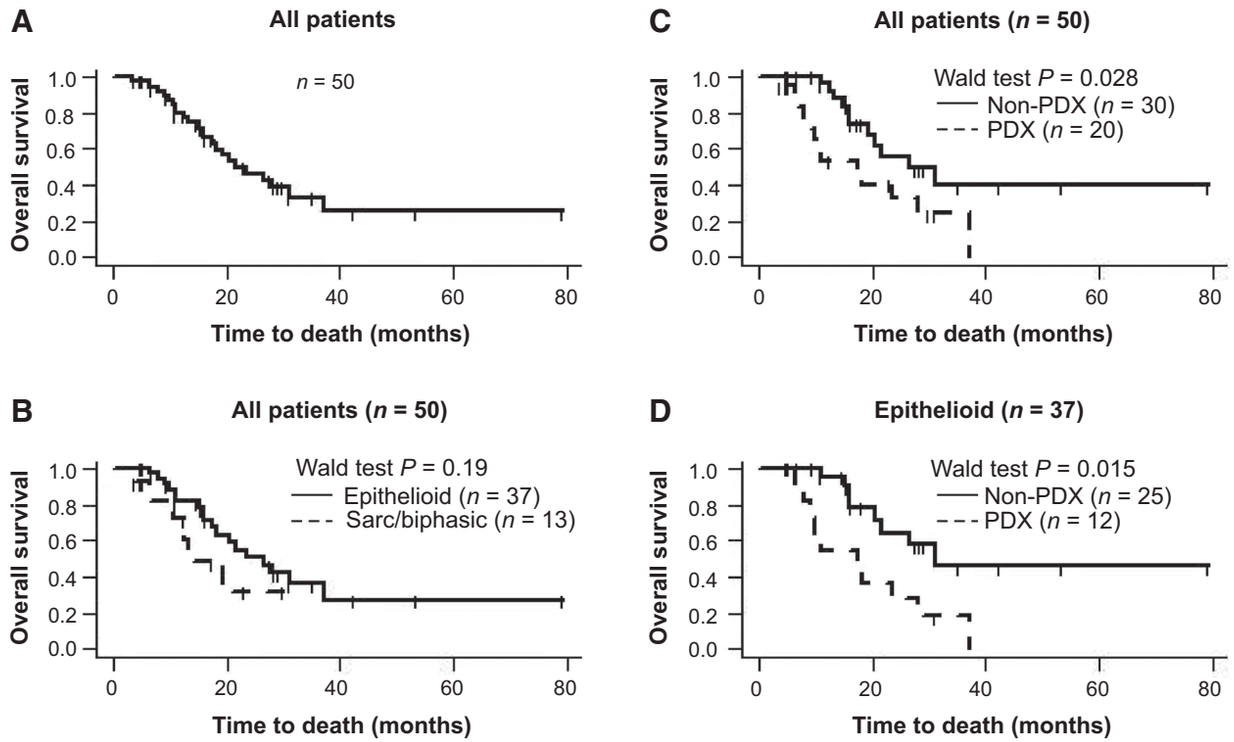


Figure 2.

Survival of MPM patients with epithelioid tumors, EPP, or preoperative chemotherapy associated with xenograft development in mice. **A,** Overall survival curve for all patients in this study. **B,** Survival of patients with xenograft development (xenograft) versus patients with xenograft failure (non-xenograft) in mice. Patients whose tumors did not engraft had significantly better survival than those that did ($P = 0.028$; HR, 2.46; CI of HR, 1.1–5.52). **C,** Survival of patients with epithelioid histology versus patients with sarcomatoid or biphasic tumors. Survival did not differ significantly between the histology types; **D,** For patients with epithelioid tumors, survival of patients with xenografts versus xenograft failure in mice. Patients with non-xenograft had significantly better survival than those with xenograft in mice ($P = 0.015$; HR, 3.2; 95% CI of ratio, 1.25–8.07).

Mesothelioma is an uncommon disease, but it is rarely resectable. As a large phase III trial demonstrated a palliative benefit to EPP only (2), fewer surgeons are offering this modality to patients, thereby limiting tissues available for functional studies. Our data suggest that small biopsies can be used, and indeed, tumors that have been exposed to cytotoxics are still feasible for PDX formation. Such an approach allows clinically relevant samples to be captured and multiple studies to be performed.

We and others have previously demonstrated the clinical utility of using these models to study response to therapies and to

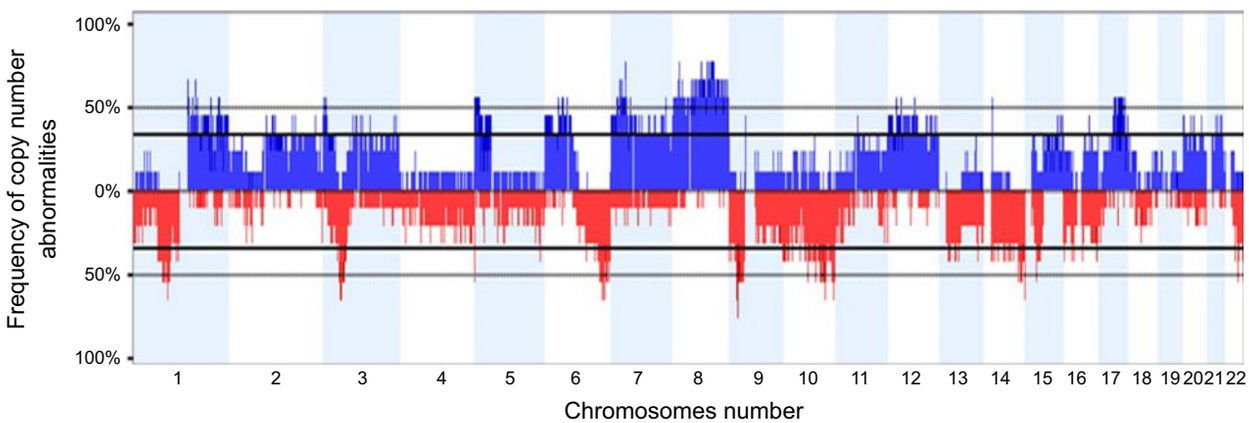


Figure 3.

Frequency of copy number abnormalities in the nine P2 mesotheliomas. Frequency of CN gains are depicted in blue bars and losses in red bars.

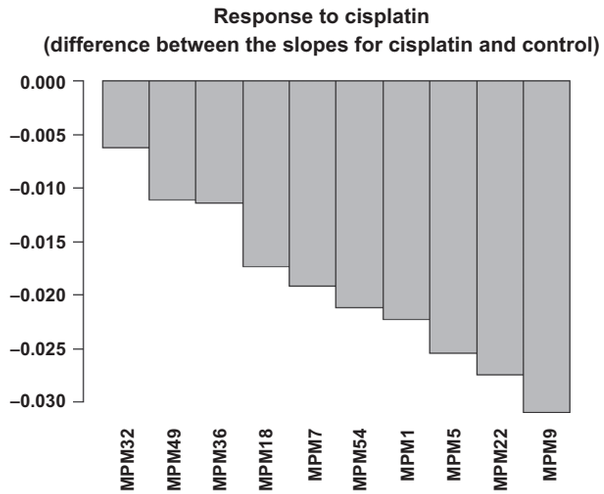


Figure 4.
The difference in growth rates between xenograft tumors treated or untreated (control) with cisplatin is shown on a log scale. Response was observed in seven of the 10 models.

develop novel agents (15, 24). In mesothelioma, a large number of phase II studies have failed and since 2003, there have been no new agents approved for the treatment of this disease. PDX models offer a more feasible approach to develop treatments and explore predictive markers. However the variability of growth and the time taken to conduct these experiments have lead many drug developers to retain the use of cell lines, which tend to grow more rapidly and more predictably. Although these problems with PDX models can be seen negatively, we believe they more accurately reflect the clinical heterogeneity of the disease than cell lines. Certainly, the large stromal component of these tumors was often retained in the PDX models, enabling functional studies targeting tumor stroma to be employed, which represents another limitation of cell line models.

Ten xenograft models were treated with cisplatin, which delayed tumor growth in most cases. Unexpectedly, pemetrexed either alone or in combination with cisplatin did not significantly impact on the growth of these xenograft models. These findings are similar to results reported by others (unpublished reports) that pemetrexed is not effective on the malignant mesothelioma SCID mouse model; *in vivo* treatment with pemetrexed (150, 250 mg/kg) did not display any effect, both on tumor growth and toxicity. Another study also demonstrated that treatment with pemetrexed did not inhibit tumor growth in a M14K tumor xenograft model. However, valproate was able to ameliorate treatment with cisplatin and pemetrexed in murine AB12 and human M38K models (25). Because of differences in folate metabolism, the use of pemetrexed in the NOD/SCID model has been reported to be ineffective (unpublished). As this drug is currently used as standard of care in MPM cases, this does represent a limitation to using PDX models in NOD/SCID mice (25).

The association of PDX formation with poorer survival also points to inherent biological differences within histologic types. Perhaps not surprisingly, most sarcomatoid and biphasic tumors engrafted, whereas only one third of the epithelioid subtype formed a PDX. In this subtype, patients whose tumors formed a PDX had a significantly poorer outcome compared with those that did not. It is well recognized that some patients with epithelioid MPM have relatively indolent disease; however, determining who these patients are *a priori* is difficult. Although some have proposed real-time PDX models to determine therapies (26), we believe such an approach is not practical for most malignancies, including MPM. However, the biological mechanisms for the differences in outcome can be better studied in the PDX model and through comparisons with the original tumor and samples from tumors that did not engraft. We have shown that high-quality DNA was retrievable in most PDX samples and able to be assayed on the OncoScan platform. Such studies would enable better differentiation of these populations with diverse clinical outcome and more importantly would enable better targeting of those with poorer prognosis.

There are limitations to these models, which should also be emphasized. Removing a tumor from the pleural cavity and placing it into the subcutaneous tissue in an immunocompromised mouse inevitably alters some factors involved in the tumor–stromal interface. However, morphologically, the tumor does retain a striking similarity to the original tumor, even at the stromal interface. It is unclear whether there is an alteration in the immunologic interface from the original tumor compared with the PDX model. Recently, two studies in MPM have shown that the PD-L1 expression is associated with sarcomatoid histology and a poorer clinical outcome (27, 28). It is possible that the PDX models also have altered immunologic phenotype given that they are also associated with a poorer clinical outcome. The NOD/SCID model also removes immunologic surveillance pressures, which the original tumor may have required, thereby altering its immunophenotype. These factors need to be carefully considered especially when considering immunologic therapies for MPM PDX models. Importantly, the immunologic differences are likely to be even greater in cell line models, where the tumor–stroma interface is absent.

We have shown that PDX models of MPM can be generated from small biopsy samples and used for functional and genomic studies. As tissue resources become more important for basic research, the passagibility and the morphologic retainment of features seen in the primary tumor make these models an important resource. Furthermore, the fact that PDXs represent a poorer clinical phenotype makes them ideal models to identify prognostically relevant markers and develop better therapeutic strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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