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Cancer Therapy: Preclinical

Clinical Cancer Research

Patient-Derived First Generation Xenografts of Non–Small Cell Lung Cancers: Promising Tools for Predicting Drug Responses for Personalized Chemotherapy

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Abstract

Purpose: Current chemotherapeutic regimens have only modest benefit for non–small cell lung cancer (NSCLC) patients. Cumulative toxicities/drug resistance limit chemotherapy given after the first-line regimen. For personalized chemotherapy, clinically relevant NSCLC models are needed for quickly predicting the most effective regimens for therapy with curative intent. In this study, first generation subrenal capsule xenografts of primary NSCLCs were examined for (*a*) determining responses to conventional chemotherapeutic regimens and (*b*) selecting regimens most effective for individual patients.

Experimental Design: Pieces ($1\times3\times3$ mm^3) of 32 nontreated, completely resected patients' NSCLCs were grafted under renal capsules of nonobese diabetic/severe combined immunodeficient mice and treated with (A) cisplatin+vinorelbine, (B) cisplatin+docetaxel, (C) cisplatin+gemcitabine, and positive responses (treated tumor area ≤50% of control, $P < 0.05$) were determined. Clinical outcomes of treated patients were acquired.

Results: Xenografts from all NSCLCs were established (engraftment rate, 90%) with the retention of major biological characteristics of the original cancers. The entire process of drug assessment took 8 weeks. Response rates to regimens A, B, and C were 28% (9 of 32), 42% (8 of 19), and 44% (7 of 16), respectively. Certain cancers that were resistant to a particular regimen were sensitive to others. The majority of responsive tumors contained foci of nonresponding cancer cells, indicative of tumor heterogeneity and potential drug resistance. Xenografts from six of seven patients who developed recurrence/metastasis were nonresponsive.

Conclusions: Models based on first generation NSCLC subrenal capsule xenografts have been developed, which are suitable for quick assessment (6-8 weeks) of the chemosensitivity of patients' cancers and selection of the most effective regimens. They hold promise for application in personalized chemotherapy of NSCLC patients. *Clin Cancer Res; 16(5); 1442–51. ©2010 AACR.*

Lung cancer is the leading cause of cancer-related mortality worldwide (1). Non–small cell lung cancer (NSCLC) represents over 80% of lung cancer deaths (2, 3). Chemotherapy has been shown to improve the survival of patients with advanced, inoperable NSCLCs or, as adjuvant therapy, to reduce the rate of relapse of patients following resection of early-stage cancers (2, 3). Generally, two-drug combinations of cytotoxic drugs such as gemcitabine, vinorelbine, and docetaxel with cisplatin or carboplatin are used. A recent meta-analysis study showed that platinum-based, adjuvant chemotherapy of patients with resected NSCLCs was associated with a 5% greater 5-year survival rate, revealing marginal effectiveness of current chemotherapeutic regimens (4). Moreover, only a portion of patients who receive first-line treatment can receive further chemotherapy because of rapid disease progression and intolerance to side effects. Additional chemotherapy is particularly limited for patients who have experienced severe toxicity with previous chemotherapy and especially for older individuals who may suffer comorbidity from effects of smoking. Clearly, optimal selection of the initial chemotherapy regimen is crucial whether it be in an advanced disease or adjuvant situation. There is an urgent need for tools to reliably and quickly predict responses of patients' cancers to particular chemotherapeutic regimens to provide more effective personalized treatment or to spare nonresponders from futile chemotherapy.

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Translational Relevance

Current chemotherapeutic regimens have only modest benefit for non–small cell lung cancer (NSCLC) patients. Cumulative toxicities/drug resistance limit the chemotherapy given after the first-line regimen, indicating a need for selecting the most effective regimens for individual patients. This requires the quick determination of chemosensitivities of individual cancers to various available regimens. Current methods using subcutaneous xenografts of patient-derived, transplantable tumor lines is not suitable due to low engraftment rates of patients' cancers and long periods required for tumor line development. We have developed a method based on the use of *first generation* cancer tissue xenografts (as distinct from established tumor lines) from patients' primary NSCLCs transplanted into the subrenal capsule graft site of immunodeficient mice. It allows the reliable and quick determination (6-8 weeks) of chemosensitivities of patients' NSCLCs. Furthermore, sufficient cancer tissue can apparently be obtained from most NSCLC patients for this assay. This approach therefore seems suitable for selecting optimal regimens for personalized chemotherapy in the clinic.

Although there is increasing evidence that responses of cancers to chemotherapy may be predicted by molecular signatures (5–8), there is currently no biomarker that can be used as a routine guide for individualized chemotherapy of NSCLC patients (8). As an alternative approach, chemosensitivity of patients' cancers to various regimens can be evaluated using transplantable subcutaneous tumor lines developed from resected cancer specimens. Although such tumor lines are valuable for drug development and may lead to useful predictions (9–12), the engraftment rate of s.c. implanted cancers is low (20-40%) and establishing transplantable tumor lines may take as long as 10 months (13–17), severely limiting the usefulness of this approach for personalized chemotherapy.

Recently, we have adopted the subrenal capsule grafting technique (18–21). Using nonobese diabetic/severe combined immune deficiency (NOD-SCID) mice, it allows very high tumor engraftment rates of various human cancers, including lung carcinoma (22–24). The subrenal capsule graft site permits high tissue perfusion and hence promotes viability and growth of the xenografts and their retention of major histopathologic and genetic characteristics of the original cancers, making them useful for drug response evaluation (22–24). In the clinic, NSCLC patients in general receive adjuvant chemotherapy 3 to 6 weeks after surgery (25–27). In view of this, the determination of drug response must be completed within this time frame. In the present study, we used *first generation* subrenal capsule xenografts of various types of primary NSCLC in NOD-SCID mice, which can be established within 2 to 4 weeks, to (*a*) determine responses of patients' NSCLCs to standard chemotherapeutic regimens, (*b*) determine the most effective regimens for individual cancers, and (*c*) check if the responses of the xenografts are correlated with clinical outcomes, indicating usefulness of the xenografts for personalized chemotherapy.

Materials and Methods

Materials and animals. Chemicals, solvents, and solutions were obtained from Sigma-Aldrich unless otherwise indicated. Six- to 8-wk-old NOD-SCID mice were bred in the Animal Resource Centre of the BC Cancer Agency Research Centre, Vancouver, Canada. Animal care and experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Patient tumor samples. Previously nontreated NSCLCs were obtained with patients' consent at Vancouver General Hospital (Vancouver, Canada). Diagnosis of NSCLC was confirmed by a pathologist (JCE or JF). Cases of complete resection were defined as those with no macroscopic residual tumor tissue (upon inspection by a thoracic surgeon, JY or KE) and a microscopically clear resection margin (as confirmed by a pathologist). Clinical outcome was tracked from follow-up visits to the surgeons, medical oncologists (NM), and the Cancer Registry. The protocol was approved by the Clinical Research Ethics Board of the University of British Columbia/BC Cancer Agency.

Drugs and regimens. Cisplatin (Mayne Pharma), vinorelbine (GlaxoSmithKline), and gemcitabine (Eli Lilly) preparations were diluted to 0.25, 0.5, and 12 mg/mL in saline, respectively. Docetaxel solutions (Aventis Pharma) were prepared with three volumes of 13% ethanol solution in distilled water and then further diluted in saline to a final concentration of 1.2 mg/mL. Drug doses and treatment schedules were obtained from published studies (28). Three cisplatin-based regimens were tested in xenografts: (A) cisplatin (2.5 mg/kg/d) + vinorelbine (5 mg/kg/d), (B) cisplatin (2.5 mg/kg/d) + docetaxel (12 mg/kg/d), and (C) cisplatin (2.5 mg/kg/d) + gemcitabine (120 mg/kg/d). Mice were treated weekly for 3 wk (i.p.). Similarly, control mice were injected with the same volumes of saline.

Tumor transplantations and treatments. Tumors were processed as outlined in Fig. 1. Within 30 min of surgical removal, excess tumor tissues not needed for pathologic diagnosis were isolated and stored at 4°C in HBSS supplemented with antibiotics. Benign and necrotic areas that were difficult to distinguish visually from viable cancer tissue were removed using an XI 120 Fluorescence Illumination System (Photonic Solutions) modified to illuminate at 436 nm with visual detection of wavelengths longer than 470 nm. Benign, necrotic, or stromal tissues fluoresced green, whereas malignant tissues fluoresced brown or brownish red (29). Tumor tissue was cut into multiple $1\times3\times3$ mm^3 pieces for grafting under renal capsules of NOD-SCID mice, as previously described (24). In the early stages of the study, it was experimentally established that the minimum volume of tissue required to test at least one

chemotherapeutic regimen (requiring four grafts per mouse, five mice per group, control and treated) should be ≥1.0 cm^2 × 0.5 cm and that the percentage of viable tumor tissue should be ≥30%. The latter was estimated by microscopic examination of eight randomly selected pieces before grafting. Xenografts were allowed to adapt to their new microenvironment in mice for 28 d. The mice were then randomly distributed into groups with at least five mice (20 grafts) per group and 3-wk drug treatments initiated with the regimens described above. All xenografts were harvested on day 49.

Histology. Each excised xenograft was bisected through the longest dimension, fixed in 10% neutral-buffered formalin, and embedded in paraffin. Twenty-three histologic sections (7 μm thick) were cut from each xenograft using a microtome and mounted on glass slides. Routine H&E staining was carried out on sections no. 1, 12, and 23. Sections 2 to 11 and 13 to 22 were retained for other purposes. For immunohistochemical staining, additional sections were cut from the paraffin blocks. The staining was carried out as previously described (24), using anti–Ki-67 (1:50) and anti–caspase-3 (1:50; DAKO) as primary antibodies.

Quantification of tumor response. Response to therapy was evaluated by measuring the tumor area in xenografts treated with one of the standard regimens compared with controls; in some cases, differences in expression of Ki-67 and caspase-3 were evaluated. H&E slides of every 11th section (section no. 1, 12, and 23, separations of 70 μm) from serially sectioned xenografts were examined independently by two pathologists (JCE and JF) to determine the proportions of viable tumor areas in treated and control grafts (% Tumor). Images of the 23rd section were captured using an AxioCam HR CCD mounted on an Axioplan 2 microscope and Axiovision 3.1 software (Carl Zeiss), with final magnifications of ×25. Images were prepared and pixel counts of each graft area were measured using Adobe Photoshop 7.0. The pixel counts were converted to true graft area (mm^2) using the formula: Graft area = Pixel counts × 2.53^2 × 10^{-6} (as established from measurements with a ruled calibration slide). The tumor areas of each graft were then calculated using the formula: Tumor area = Graft area × %Tumor. Response to treatment was determined by the comparison of residual, viable tumor areas in treated groups with their controls. A significantly smaller tumor area (≤50% of control; $P < 0.05$) was considered a positive response.

For quantification of immunostaining of cells by Ki-67 and caspase-3, five randomly selected high-power (×400) images from each graft were captured as described above. Ki-67– or caspase-3–positive cells and total cells (positive + negative) were counted using Adobe Photoshop 7.0 and the NIH

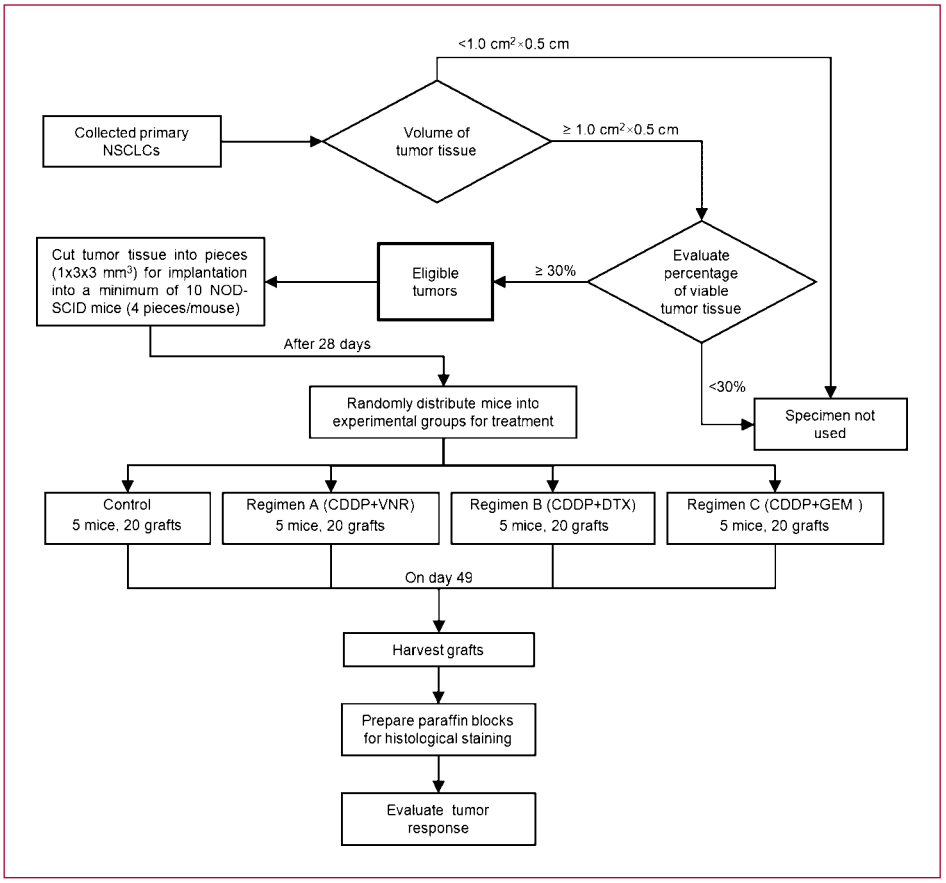

Fig. 1. Establishment of first generation subrenal capsule xenografts of nontreated primary NSCLCs and their treatment with standard chemotherapeutic regimens. CDDP, cisplatin; VNR, vinorelbine; DTX, docetaxel; GEM, gemcitabine.

ImageJ software. The percentages of Ki-67– or caspase-3–positive cells (Ki-67 or caspase-3 index) were calculated using the formula: Index = number of positive cells × 100/number of total cells.

Statistical analysis. Viable tumor areas of the treated and control groups were averaged and presented as means ± SD. *P* values were calculated using a permutation test of the means (30). Data of Ki-67 and caspase-3 indices are presented as means ± SD and analyzed by the Student's *t* test. Results with *P* values <0.05 were considered significant in all of our analyses.

Results

Establishment of xenografts from NSCLC tissue. First generation xenografts from 32 completely resected NSCLCs were established following the procedures outlined in Fig. 1. The characteristics of the patients are presented online. In total, 1,573 xenografts were generated. Of the 527 grafts assigned to the nontreated (control) groups, 476 grafts survived resulting in an engraftment rate of 90%.

Retention by xenografts of biological characteristics of the original cancers. As revealed by microscopic examination, the first generation xenografts in the control groups (no chemotherapy) closely resembled the original cancers with the retention of characteristic morphologic and immunohistochemical features (Figs. 2A, B, and D and 3A, B, and D). Xenografts from differentiated squamous cell carcinomas expressed squamoid features, such as keratinization and intercellular bridges, as found in the original cancers. Similarly, glandular structures were found in xenografts of moderately differentiated adenocarcinomas; lepidic growth patterns were found in grafts of bronchoalveolar

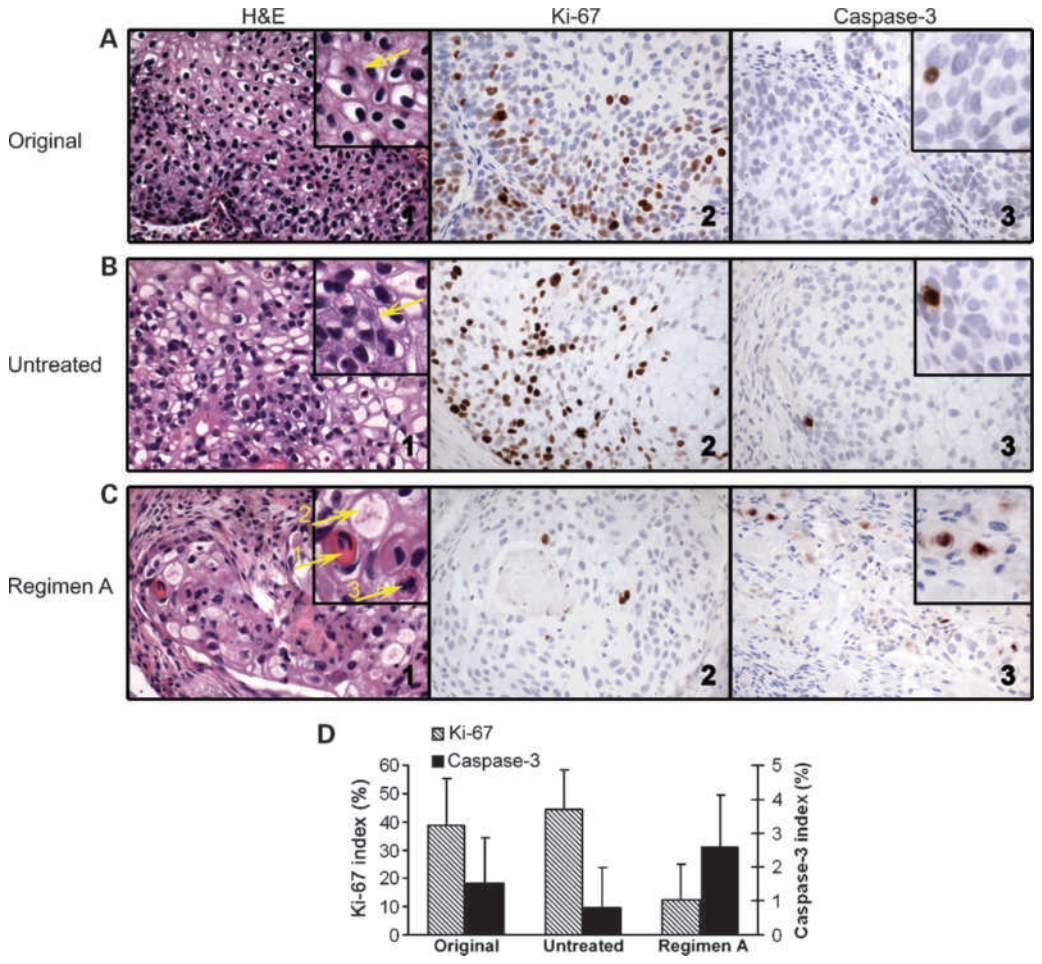

Fig. 2. Tissue sections of a chemoresponsive tumor (case 05L29). A, patient's squamous cell carcinoma showing intercellular bridges (arrow). B, nontreated first generation grafts showing intercellular bridges (arrow), keratinization, and stratification. C, regimen A (cisplatin+vinorelbine)–treated grafts showing fewer viable tumor cells with eosinophilic (arrow 1) or clear foamy (arrow 2) cytoplasm, swollen nuclei with chromatin clumping (arrow 3), and reduced Ki-67 and increased caspase-3 expression. D, Ki-67 and Caspase-3 indices in controls are similar to those in patient's original tumor ($P > 0.05$). Regimen A induced a decrease in Ki-67 and an increase in Caspase-3 indices ($P < 0.01$); original magnification of insets, ×400.

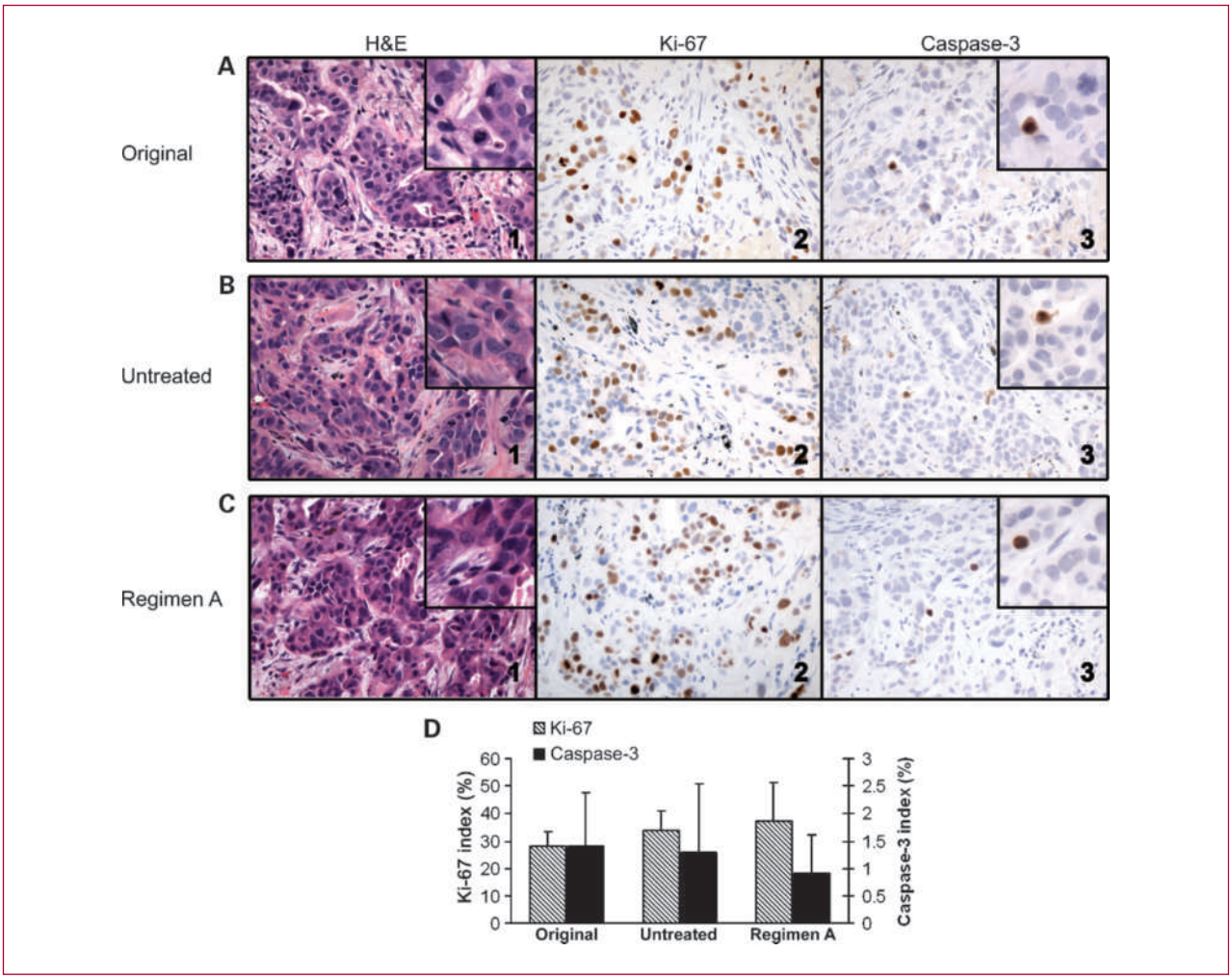

Fig. 3. Tissue sections of a nonresponsive tumor (case 05L19). A, patient's adenocarcinoma. B, nontreated first generation grafts showing clusters of cancer cells with abortive glandular structures similar to those in the original cancer. C, regimen A (cisplatin+vinorelbine)–treated grafts showing few histologic changes compared with controls. Ki-67 and caspase-3 expressions are similar to those in the original tumor and the controls. D, Ki-67 and caspase-3 indices in controls were similar to those in the patient's original tumor ($P > 0.05$). Regimen A–induced changes in Ki-67 and casepase-3 indices were not significant ($P > 0.05$). Original magnification of insets, ×400.

and some mixed subtype adenocarcinomas; and polygonal cell nests were found in grafts of large cell carcinomas. There was no significant change in expression of Ki-67 and caspase-3 in either parent or engrafted tumors.

Tumor responses to chemotherapeutic regimens. Sixteen of the 32 tumors provided sufficient tissue for testing all three chemotherapeutic regimens (Table 1). All of the remaining tumors were tested with regimen A, the current evidence-based regimen for adjuvant therapy, and three with regimen B. Positive tumor responses (in bold) were defined by a significantly smaller tumor area of the treated xenografts (≤50% of control; $P < 0.05$). The tumor response rates to regimens A, B, and C were 28% (9 of 32), 42% (8 of 19), and 44% (7 of 16), respectively.

Of the 16 cases tested with all three regimens, 19% (3 of 16) were responsive to all three treatments; 50% (8 of 16) were responsive to only one or two of the regimens; and 31% (5 of 16) were nonresponsive to any of the regimens (Table 1). The pattern of sensitivity to the three regimens is illustrated as a Venn diagram presented online. The data suggest that approximately two thirds (69%) of the NSCLC patients could respond to at least one of the three standard regimens, and that one third (31%) of the patients would need treatments other than regimens A, B, and C. It may be noted that some cancers that were resistant to a particular regimen were found to be sensitive to other regimen(s). This indicates the usefulness of the xenografts for identifying differences in chemosensitivity of individual cancers.

Histologic changes after chemotherapy were obvious in responding tumors (e.g., case 05L29, Fig. 2). In the grafts of such tumors, the majority of tumor tissue was replaced by scar tissue or necroses. The remaining cancer cells were

Table 1. Xenograft responses to chemotherapeutic regimens

Case	Tumor area (mm^2) after treatment			
	Control	Regimen A*	Regimen B*	Regimen C*
05L2	1.80 ± 1.42	***0.35 ± 0.27***†,‡	***0.54 ± 0.54***†,‡	***0.78 ± 0.55***‡,§
05L42	4.93 ± 3.41	***0.66 ± 0.85***†,‡	***0.93 ± 0.89***†‡	***2.11 ± 2.54***†,‡
06L28	0.83 ± 0.83	***0.01 ± 0.02***†	***0.05 ± 0.06***†	***0.02 ± 0.03***†
L7	1.90 ± 1.60	***0.35 ± 0.23***†	1.77 ± 1.29	1.15 ± 1.25
L12	1.50 ± 1.48	1.19 ± 1.34	***0.58 ± 0.71***‡,§	1.21 ± 1.58
05L1	0.71 ± 0.73	0.48 ± 0.60	0.76 ± 0.84	***0.20 ± 0.28***‡,§
05L3	1.01 ± 0.69	0.58 ± 0.53	***0.19 ± 0.16***†,‡	***0.42 ± 0.40***‡,§
05L8	1.09 ± 2.30	0.02 ± 0.04§	***0.03 ± 0.07***§	0.05 ± 0.05
05L48	1.06 ± 0.86	***0.64 ± 0.48***	***0.37 ± 0.47***†,‡	0.61 ± 0.94
05L53	1.83 ± 1.66	***0.61 ± 0.42***‡,§	1.07 ± 2.03	***0.69 ± 0.43***†,‡
06L13	2.51 ± 2.07	3.76 ± 3.54	1.47 ± 2.33	***0.78 ± 1.30***†,‡
05L39	0.79 ± 1.33	0.44 ± 0.66	0.57 ± 0.67	0.27 ± 0.33
05L44	0.79 ± 2.02	0.37 ± 0.99	0.17 ± 0.36	0.19 ± 0.36
05L49	0.24 ± 0.36	0.24 ± 0.26	0.12 ± 0.19	0.22 ± 0.31
06L12	0.04 ± 0.05	0.03 ± 0.03	0.06 ± 0.10	0.06 ± 0.07
06L15	0.20 ± 0.12	0.15 ± 0.05	0.21 ± 0.18	0.32 ± 0.23
L15	1.57 ± 2.48	***0.20 ± 0.54***†,‡	0.45 ± 0.94	
05L32	0.34 ± 0.46	0.03 ± 0.05	***0.05 ± 0.07***†	
05L43	0.84 ± 1.49	0.18 ± 0.44	0.23 ± 0.47	
L4	7.39 ± 5.51	7.74 ± 5.09		
05L14	0.64 ± 1.55	0.77 ± 1.04		
05L18	0.01 ± 0.05	0.15 ± 0.53		
05L19	0.30 ± 0.20	0.22 ± 0.10		
05L21	1.35 ± 0.62	0.88 ± 0.77		
05L24	0.16 ± 0.22	0.06 ± 0.10		
05L25	0.20 ± 0.22	***0.05 ± 0.07***‡,§		
05L27	0.26 ± 0.22	0.21 ± 0.18		
05L29	3.52 ± 2.12	***0.15 ± 0.14***†		
05L31	0.22 ± 0.20	0.12 ± 0.15		
05L35	0.55 ± 1.05	0.07 ± 0.08		
06L1	0.05 ± 0.06	0.05 ± 0.05		
06L2	2.46 ± 4.67	0.73 ± 0.59		
Response rate	(n = 32)	28% (9/32)	42% (8/19)	44% (7/16)

*Regimen A, cisplatin+vinorelbine; regimen B, cisplatin+docetaxel; regimen C, cisplatin +gemcitabine. Data in bold indicate significant responses.

$^{\dagger}P < 0.01$.

‡Indicating individual grafts containing a high proportion of residual viable tumor cells (>50% of tumor area).

$^{\S}P < 0.05$.

generally characterized by swollen nuclei, clumped chromatin, and eosinophilic or foamy cytoplasm (Fig. 2C1). Mitotic figures were rare. Immunohistochemical staining with anti–Ki-67 and anti–caspase-3 antibodies indicated reduced cell proliferation and increased apoptosis (Fig. 2C2-3 and D). In contrast, cancer cells in nonresponding tumors (e.g., case 05L19, Fig. 3) had few histologic changes compared with controls (Fig. 3C1) and expression of Ki-67 and active caspase-3 was not significantly affected by the chemotherapy (Fig. 3C2-3 and D).

It was found that some tumors that were responsive to chemotherapy also contained nonresponsive subpopulations (e.g., case 05L53, Fig. 4). Thus, although 9 of 12 grafts of the 05L53 tumor responded to regimen A, showing a significant overall decrease in tumor growth with the entire or majority of the grafts replaced by scar tissue (Table 1; Fig. 4B and C), 3 grafts contained a high proportion of morphologically viable cancer cells (>75% by area, Fig. 4D) similar to the controls (Fig. 4A). Furthermore, six of nine cases responding to regimen A, five of eight cases responding to regimen B, and six of seven cases responding to regimen C showed foci of apparently viable cancer cells (Table 1). The presence of such nonresponding tumor subpopulations in responsive tumors illustrates

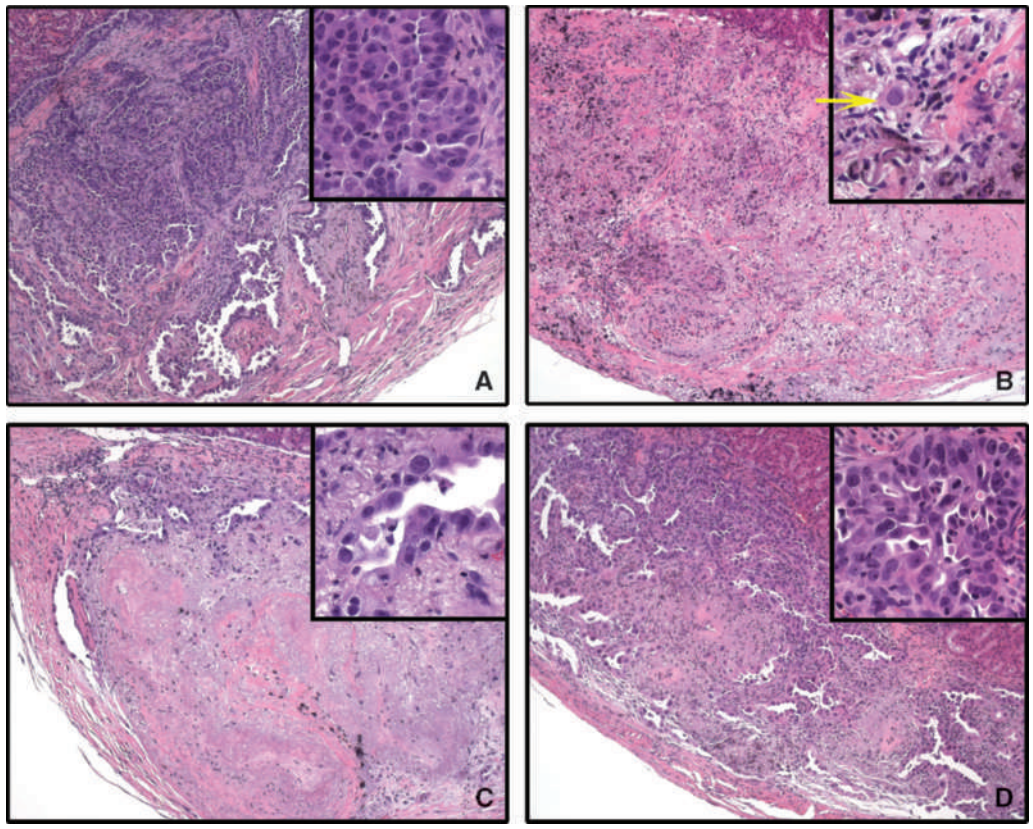

Fig. 4. Tissue sections of a chemosensitive tumor containing drug-resistant subpopulations (case 05L53). A, section of a nontreated adenocarcinoma graft. B to D, grafts treated with regimen A (cisplatin+vinorelbine). B and C, major replacement of tumor cells by fibrous scar tissue. Single residual tumor cells with swollen nuclei (arrow) or small clusters of residual tumor cells with unilaminar glandular structure were spotted. D, presence of a high proportion of viable-looking residual tumor cells (>75% by area) with few histologic changes indicative of drug resistance. Insets, magnified areas (original magnification, ×400).

tumor heterogeneity as a basis for potential drug resistance and recurrence.

Comparison of tumor responses in mice with clinical outcome of patients. The chemotherapeutic response of xenografts of primary NSCLCs was compared with the available clinical outcome of donor patients (disease-free time, tumor recurrence, metastasis) treated with a matching adjuvant chemotherapeutic regimen and followed up for a minimum of 2 years (Table 2). Among the 32 original tumors entered into the study, 11 patients had adjuvant chemotherapy that matched a regimen used for the xenografts, i.e., regimen A: vinorelbine+cisplatin (80 mg/m^2 cisplatin, day 1, 30 mg/m^2 vinorelbine, days 1 and 8, a 21-day cycle for four cycles).[10] It was found that xenografts from six of seven patients, who developed recurrence/metastasis during the 2-year follow-up, were nonresponsive in mice. For four patients showing no recurrence (all stage IB), there was no correlation between clinical outcome and responsiveness of their xenografts, probably related to complete removal of these early-stage cancers by surgery.

Discussion

Experimental *in vivo* models that closely mimic the biology of NSCLCs in patients are urgently needed to reliably determine optimal chemosensitivities of individual cancers for personalized chemotherapy (31, 32). Such models would also have to allow testing of drugs within a short time frame (6-8 weeks) to conform with timely initiation of therapy (25–27). The first generation xenografts of primary NSCLCs used in the present study were shown to fulfill these criteria. The high engraftment rate (90%) obtained by subrenal capsule grafting allowed the survival of the various types of NSCLC examined. The high take rate contrasts with the much lower rates (20-40%) reported for subcutaneous graft models (13–17) and is likely a result of superior nutrient supply and enhancement of graft microvascularity provided by the subrenal capsule site (18–24). The high engraftment rate was further ensured by rigorous selection of viable tumor tissue through fluorescence illumination and

[10] Management manual, http://www.bccancer.bc.ca

Table 2. Comparison of clinical outcome of patients (postoperatively treated with regimen A) with responses of their tumors to regimen A in mice

Case	Patients				Xenografts
	Histopathology	pTNM stage	Clinical outcome	Disease-free time (mo)	Response to regimen A
05L3	AC	IIIB	Recurrence	2.4	NR
05L18	AC	IIB	Recurrence	15.87	NR
06L13	SCC	IIIB	Recurrence	23.77	NR
06L15	AC	IIIB	Recurrence	11.03	NR
L12	AC	IIIA	Metastasis	4.73	NR
05L31	LCC	IIB	Metastasis	10.63	NR
05L53	AC	IIB	Recurrence	11.4	R
06L2	AC	IB	No recurrence	>24.00	NR
06L12	AC-SCC	IB	No recurrence	>24.00	NR
L15	AC	IB	No recurrence	>40.00	R
05L29	SCC	IB	No recurrence	>38.07	R

NOTE: Regimen A, vinorelbine+cisplatin

Abbreviations: pTNM: pathologic tumor-node-metastasis; AC, adenocarcinoma; NR, nonresponding; SCC, squamous cell carcinoma; LCC, large cell carcinoma; R, responding.

by identifying such tissue in cryostat sections from randomly selected pregraft fragments. The first generation xenografts also closely resembled the original cancers with regard to type-specific morphologic and immunohistochemical features (Figs. 2 and 3), as well as tumor heterogeneity, as indicated by the presence of subpopulations with different sensitivities to treatment (Fig. 4). Furthermore, they contained a microenvironment similar to that of the patients' cancers since original human tumor stroma is still present in early xenograft generations (24). Our previous studies have shown that even transplantable tumor tissue lines, developed through months of serial subrenal capsule grafting, were highly similar to the original cancers with respect to phenotypic as well as genotypic properties (22–24). Clearly, the first generation xenografts used in this study are closely related to the original malignancies, and models based on such xenografts for predicting drug responses should be highly relevant to the clinical situation.

As an alternative, use of orthotopic xenograft models could be considered. A major advantage of such models is that they allow studying mechanisms of tumor metastasis and tumor-stroma interactions in a more clinically relevant site (33, 34). However, orthotopic engraftment of lung cancer can be associated with lower tumor take rates (35) leading to potential loss of cancer subpopulations and hence of predictive value of the models. As well, host mortality is high as a result of invasive thoracotomy required for this procedure (34). In the present study, we therefore adopted the subrenal capsule grafting method that has a higher engraftment rate and is easier to implement. In contrast to subrenal capsule assays conducted by other groups (20, 21) who started treatment right after transplantation of cancer tissue (for 6-11 days), we allowed the xenografts to stabilize for 4 weeks before treatment with the drugs. This enhances the development of microvasculature in the xenografts, allowing delivery of drugs to the cancer cells in a manner similar to that occurring during chemotherapy in the clinic.

The entire process of establishing xenografts from a patient's tumor in immunodeficient mice and testing their response to chemotherapeutic drugs was completed within 8 weeks (4 weeks required for adaptation, 3 weeks for chemotherapy, and 1 week for data analysis). Recent histologic observations in our laboratory[11] have indicated that the adaptation period could be as short as 2 weeks, reducing the entire process to 6 weeks. This relatively short period of 6 to 8 weeks makes the first generation xenografts, as distinct from transplantable tumor lines (13, 15, 24, 36), suitable for clinical application.

For personalized chemotherapy, one would ideally like to test the chemosensitivity of an individual cancer using at least three different chemotherapeutic regimens. We have empirically determined that it would require 2 cm^2 × 0.5 cm of tumor tissue and ≥30% viable tumor cells (using one common control, see Fig. 1). The vast majority of NSCLC patients in which adjuvant chemotherapy is indicated according to the tumor-node-metastasis stage would have primary tumor diameters of >2 cm and provide sufficient tumor tissue for standard pathologic examination as well as tests of a variety of regimens using the subrenal capsule xenograft method.

The response rates of the xenografts to individual chemotherapeutic regimens, which varied from 28% (regimen A: cisplatin+vinorelbine) to 42% (regimen B: cisplatin+docetaxel) to 44% (regimen C: cisplatin+gemcitabine; Table 1), are close

[11] Y.Z. Wang et al., unpublished observations.

to those reported in clinical trials (37–39). The chemotherapy-induced histologic changes in the xenografts (Fig. 2) are consistent with those documented in clinical studies examining NSCLC specimens following preoperative chemotherapy (40–43). Furthermore, some responding tumors contained substantial proportions of viable tumor cells (Fig. 4), indicative of drug resistance and a potential for tumor recurrence as it frequently occurs in patients after a partial or even a complete response (37, 38, 44). Importantly, the finding that the xenografts could be used to identify differential chemosensitivities of individual cancers indicates that the approach is suitable for the selection of optimal regimens for personalized chemotherapy. If validated, this technique could identify a side population of patients for whom standard regimens are futile. As shown in the Venn diagram (online), ~31% of the tumors were responsive to none of the three standard regimens, confirming that novel drug treatments rather than standard chemotherapeutic regimens are critically needed for the successful treatment of these NSCLCs. The xenograft approach may also be useful for predicting the patients' outcome, given that a correlation was found between recurrence/metastasis in patients and nonresponsiveness of their tumor xenografts in mice. However, in view of the small number of patients used, further studies are required.

In conclusion, models based on first generation NSCLC subrenal capsule xenografts have been developed that are suitable for quick assessment of chemosensitivity of individual patients' cancers and selecting the most effective therapeutic regimens. They hold promise for application in personalized chemotherapy of NSCLC patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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