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Modulation of tumor cell response to chemotherapy by the organ environment

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Abstract

The outcome of cancer metastasis depends on the interaction of metastatic cells with various host factors. The implantation of human cancer cells into anatomically correct (orthotopic) sites in nude mice can be used to ascertain their metastatic potential. While it is clear that vascularity and local immunity can retard or facilitate tumor growth, we have found that the organ environment also influences tumor cell functions such as production of degradative enzymes. The organ microenvironment can also influence the response of metastases to chemotherapy. It is not uncommon to observe the regression of cancer metastases in one organ and their continued growth in other sites after systemic chemotherapy. We demonstrated this effect in a series of experiments using a murine fibrosarcoma, a murine colon carcinoma, and a human colon carcinoma. The tumor cells were implanted subcutaneously or into different visceral organs. Subcutaneous tumors were sensitive to doxorubicin (DXR), whereas lung or liver metastases were not. In contrast, sensitivity to 5-FU did not differ between these sites of growth. The differences in response to DXR between s.c. tumors (sensitive) and lung or liver tumors (resistant) were not due to variations in DXR potency or DXR distribution. The expression of the multidrug resistance-associated P-glycoprotein as determined by flow cytometric analysis of tumor cells harvested from lesions in different organs correlated inversely with their sensitivity to DXR: increased P-glycoprotein was associated with overexpression of *mdr*1 mRNA. However, the organ-specific mechanism for upregulating *mdr*1 and P-glycoprotein has yet to be elucidated.

Introduction

Despite significant improvements in diagnosis, surgical techniques, general patient care, and local and systemic adjuvant therapies, most deaths from cancer are due to metastases that are resistant to conventional therapies. In a large number of patients with cancer, metastasis may well have occurred by the time of diagnosis. The metastases can be located in different lymph nodes and visceral organs and in various regions of the same organ, thus complicating their treatment. Furthermore, the specific organ environment can modify the response of a metastatic tumor cell to systemic therapy and alter the efficiency of anticancer agents [1].

The major barrier to the treatment of metastases is the biological heterogeneity of cancer cells in primary and secondary neoplasms. This heterogeneity is exhibited in a wide range of genetic, biochemical, immunological, and biological characteristics, such as cell surface receptors, enzymes, karyotypes, cell morphologies, growth properties, sensitivities to various therapeutic agents, and ability to invade and produce metastasis [1–5].

The search for the mechanisms that regulate the pattern of metastasis began in 1889 when Stephen

Paget asked 'what is it that decides what organs shall suffer in a case of disseminated cancer?' [6]. Paget's inquiry was motivated by the discrepancy between blood flow and relative frequency of metastases in different organs. He examined the autopsy records of women who died of breast cancer and patients with other neoplasms and concluded that the pattern of metastasis was predictable. He drew attention to the frequency of ovarian metastases and to the differences in incidence of skeletal metastases from different primary tumors. These findings were not compatible with the view that the pattern of metastasis was due to 'a matter of chance' or that tissues behaved passively in determining the probability of clinically relevant metastases. Rather, Paget concluded that certain favored tumor cells (the 'seed') had a specific affinity for growth in the milieu provided by certain organs (the 'soil'). Metastasis resulted only when the 'seed and soil' were compatible [6].

A modern definition of this hypothesis consists of three principles. First, neoplasms are heterogeneous in biologic and metastatic properties [7]. Second, the process of metastasis is not random. Rather, it consists of a series of linked, sequential steps that must be completed by tumor cells if metastases are to develop [8]. Thus, metastatic cells must succeed in invasion, embolization, survival in the circulation, arrest in a distant capillary bed, and extravasation into and multiplication in organ parenchyma. Although some of the steps in this process contain stochastic elements, metastasis as a whole favors the survival and growth of a few subpopulations of cells that preexist within the parent neoplasm [7]. Metastases can have a clonal origin, and different metastases produced from the same primary neoplasm can originate from the proliferation of different single cells [9, 10]. Third, the outcome of metastasis depends on the interaction of metastatic cells with different organ environments [11]. Thus, both the 'seed' and the 'soil' profoundly influence the outcome of systemic therapy for cancer.

While a great deal of attention has been given to the heterogeneous nature of neoplasms, which includes variations in intrinsic sensitivity to chemotherapeutic agents [2, 12], less emphasis has been given to the influence of the organ environment, i.e., the site of growth, on tumor response to anticancer agents [5, 13, 14]. This issue is important because it is not uncommon to observe the regression of cancer metastases in one organ and their continued growth in others after systemic therapy [15, 16]. In a classic study, Slack and Bross [17] analyzed data from drug-screening trials with 1687 neoplasms growing in 6 organ sites for primary neoplasms and 6 organ sites for their metastases. Sixty days after chemotherapy, the percentage reduction in tumor size differed significantly among different metastases growing in different organs, but among primary tumor sites it did not. With few exceptions, lymph node and skin metastases were more susceptible to chemotherapy than metastases in visceral organs [17]. Differences in sensitivity to various chemotherapeutic agents of experimental tumors growing in different organs have also been reported by several investigators [12, 18, 19]. Tumors in the subcutis were more sensitive than tumors growing in visceral organs, agreeing with clinical observations [14–16]. The nutritional status of cells [20], presence of growth factors and other signal-transducing agents [21], oxygenation [20–23], pH [24–26], extent of vascular network and its functionality [27–31], and local immunity [32] can all contribute to the success or failure of cancer therapy.

Several intrinsic properties of tumor cells can render them resistant to chemotherapeutic drugs [33]. These could include an amplification of the *mdr*1 gene and overexpression of the M_r 170,000 surface P-glycoprotein (P-gp) [34–39], overexpression of the M_r 22,000 calcium-binding cytoplasmic protein [40, 41], increased glutathione transferase levels [42], altered cellular calcium and calmodulin levels [43, 44], formation of double-minute chromosomes [45], increased activity of protein kinase C (PKC) [46, 47], and lack of drug interference with type II topoisomerase activity [48, 49].

The intrinsic resistance of tumor cells to chemotherapeutic drugs can be mediated by both genetic and epigenetic mechanisms. The former can develop through gene amplification (notably, the *mdr*1 gene), gene rearrangements, and transcriptional, translational, and posttranslational events [50]. Phenotypic changes that can modulate drug resistance are often associated with increased activities

of drug-detoxifying enzymes [51], metabolism-regulating enzymes, and efflux proteins [52]. In addition, increased levels of PKC activity have been observed in several MDR tumor cell lines [46, 47]. Similar effects have also been associated with decreased activity of specific enzymes or influx of transmembrane proteins and production of altered enzymes with decreased affinity for a given drug.

Most of the above data have been derived from examining tumor cells growing in culture. However, the relevance of culture conditions to the clinical reality is unclear. The object of this review is to summarize several relevant experimental metastasis systems that clearly demonstrate the profound influence of the organ environment on the response of tumor cells to systemic therapy.

Site-dependent differences in response of the UV-2237 murine fibrosarcoma to systemic therapy with doxorubicin

To study the influence of organ microenvironment on the response of metastatic cells to systemic chemotherapy, we inoculated the murine UV-2237MM fibrosarcoma cells into different organs of syngeneic C3H/HeN mice and followed this with i.v. administration of DXR [53]. Focal 'primary' tumors growing in the subcutis or spleen were sensitive to DXR, whereas experimental metastases in the lung were not.

The mechanisms that regulate this differential response to DXR are unclear, but tumor vascularity can influence the delivery of drugs to a tumor [27–31], and blood flow to tumors is not regulated by the same mechanisms operative for normal tissues [15, 18]. However, when we measured blood supply to tumors and organs by monitoring the distribution of ^{51}Cr-labeled RBC after i.v. injection, we found no correlation between this measurement and response to DXR, ruling out a simple difference in vascularity as a controlling factor in this process.

Differences in accumulation of DXR in tumors growing at different sites could also account for differences in antiproliferative responses. Our results, however, indicated that the DXR concentration in the lung was at least twice that in the skin or spleen [53]. The higher distribution of DXR to lung metastases than to skin tumors agreed with previous results in which the uptake of DXR [16] or cyclophosphamide [54] was increased in lung metastases produced by intramuscularly growing rodent sarcomas. Thus, the accumulation of DXR in the murine fibrosarcoma lesions did not correlate with their sensitivity to the drug.

The DXR resistance of fibrosarcoma lung metastases was likewise not due to the emergence of a resistant subpopulation of cells from this heterogeneous neoplasm [55]. We base this conclusion on an examination of DXR sensitivity under *in vitro* conditions. Although in some tumor systems, cells from lung metastases have been shown to be more sensitive to chemotherapy than parental cells, in the case of the UV-2237 fibrosarcoma, DXR sensitivity was similar in both parental cells and cells isolated from lung metastases.

PKC expression can be altered by tumor-promoting phorbol esters and by oncogenic transformation [46, 47], providing evidence that growth factors and related agents may serve as paracrine factors that alter PKC expression in particular organ environments [56]. Since PKC activity levels correlate with DXR resistance in the UV-2237 fibrosarcoma cell line and its DXR-selected multidrug resistant variants [27], we examined PKC activity levels in tumors growing at different organ sites. Similar PKC activity levels in lung, spleen, and subcutaneous tumors were found, indicating that these organ environments did not alter PKC expression in the fibrosarcoma cells.

Resistance to chemotherapy, such as alkylating agents, can develop in tumors by mechanisms that are operative only *in vivo* [18]. Some of these mechanisms can involve tissue pH [24–26], oxygenation [20–23, 57–61], local immunity, cytokines, and other inhibitors of tumor cell growth. These may be additive or antagonistic to one another or to chemotherapeutic agents. In particular, the role of oxygen in cell killing by chemotherapeutic drugs [21, 22, 57–61] and in cell proliferation has been extensively studied in different *in vitro* systems [58–61], including growth in semisolid agar. DXR was found to be significantly more toxic to hypoxic cells. The influence of oxygen tension, however, appears to be tis-

sue type-dependent. Cells from pancreas and ovarian carcinoma grew well in 5% or lower oxygen atmosphere, whereas lung cancer cells grew better in 20% oxygen atmosphere [61]. In the case of ovarian carcinomas, incubation in a reduced oxygen atmosphere increased tumor sensitivity to DXR. These differences in oxygen requirement could represent the physiological oxygen tension for tumor cells *in situ*. Although many mammalian tissues have a pO_2 equivalent to a 5% oxygen atmosphere, cells of the pulmonary system require higher oxygen tension [62].

DXR and other quinone-containing compounds are capable of reacting with molecular oxygen to generate various oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radicals [63]. The intracellular production of these toxic radicals by DXR has the potential to produce cytotoxicity, but the effects of free radicals is neutralized by antioxidant enzymes such as superoxide dismutase [64], which is activated by hyperoxia [65]. All these factors could combine to produce the present results: tumors growing in the lungs are bathed by oxygen and are resistant to DXR, whereas tumors growing in the subcutis and the spleen grow under relatively anoxic conditions favorable to the antiproliferative effects of DXR [53, 66].

These studies have shown that growth in the lung renders fibrosarcoma cells relatively resistant to systemic administration of DXR and that variations in oxygenation may well be the cause. Whether growth in other visceral organs also produces these effects remains to be examined.

The effects of the organ environment on sensitivity of colon carcinoma to chemotherapy

Human colon carcinoma

Human colon carcinomas are heterogeneous for a variety of biologic properties that include invasion and metastasis. The presence of a small subpopulation of cells with a highly metastatic phenotype has important clinical implications for diagnosis and therapy of cancer. For this reason, it is important to develop animal models for the selection and isolation of metastatic variants from human neoplasms and for testing the metastatic potential of human tumor cells [67].

We have implanted human colon cancer cells (obtained from a surgical specimen) into different organs of nude mice and then recovered the tumors and established each in culture [67–69]. The colon cancer cells implanted into the subcutis of nude mice produced local tumors with only limited invasiveness. This lack of invasion, as well as the consequent lack of metastasis, has often been associated with the development of a dense, fibrous capsule around the tumor [70]. One tumor cell property that is a prerequisite for metastasis is the ability to degrade connective-tissue extracellular matrix and basement membrane components that constitute barriers against invading tumor cells [71]. Metastatic tumor cells possess various proteases and glycosidases capable of degrading extracellular matrix-degrading enzymes such as type IV collagenase (gelatinase, matrix metalloproteinase 2) and heparinase (heparan sulfate-specific endo-β-D-glucuronidase) in metastatic tumor cells. We found a strong correlation between the type IV collagenase activity of human colon carcinoma cells and their ability to metastasize to the liver after the cells were inoculated into the spleen of nude mice [72].

We examined the influence of organ environment on the metastasis of human colon carcinoma cells and on their extracellular matrix-degrading activities using four different cell lines with different metastatic potentials. When the cells of each line were injected subcutaneously, none produced any visceral metastases. In contrast, when they were injected into the cecum, they metastasized to regional mesenteric lymph nodes and the liver [72].

Since the interaction of stromal fibroblasts can influence the tumorigenicity and biological behavior of tumor cells, we determined whether organ-specific fibroblasts could directly influence the invasive ability of human colon carcinoma cells [73]. Primary cultures of nude mouse skin, lung, and colon fibroblasts were established. Invasive and metastatic cells were cultured alone or with the fibroblasts. Growth and invasive properties of the cancer cells were evaluated as was their production of gelatinase activity. Colon carcinoma cells grew on

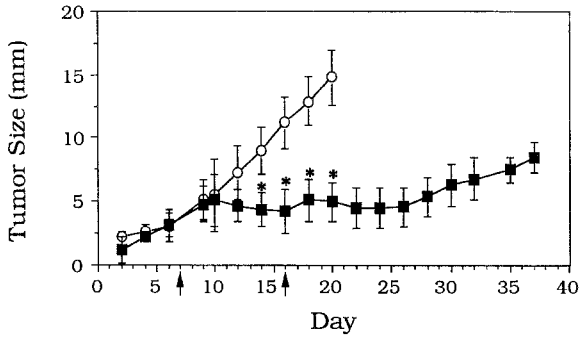

Fig. 1. Response of human colon carcinoma cells growing in the nude mouse s.c. tissue to DXR. Cells were injected into groups of nude mice (n = 10). DXR was given i.v. on days 7 and 16 at a dose of 10 mg/kg (arrows). Tumors were measured every other day in 2 diameters, and the average was taken. Control mice (○); DXR-treated mice (■). * $P < 0.05$.

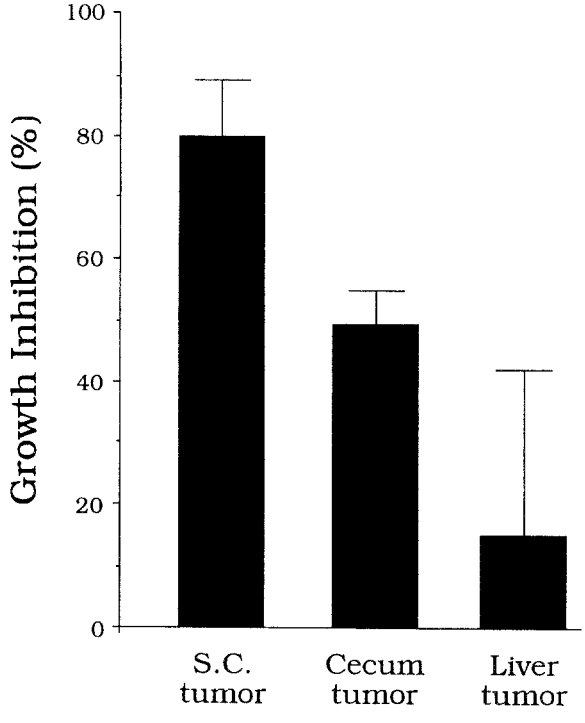

Fig. 2. Response of human colon carcinoma cells growing in different organs to DXR. Cells were injected s.c. into the spleen in order to produce liver metastases or into the cecal wall of nude mice. DXR was given on days 7 and 16 at 10 mg/kg. Mice with s.c. and liver tumors were killed 22–28 days after tumor cell injection, and mice with cecal tumors 28–35 days after. Tumors were weighed; livers with colon cancer metastases were weighed and the average weight of normal livers was subtracted to derive tumor weight. The data shown are the mean inhibition of tumor growth ± SEM (10 mice/group).

monolayers of all 3 fibroblast cultures but did not invade through skin fibroblasts. Cancer cells growing on plastic and on colon or lung fibroblasts produced significant levels of latent and active forms of type IV collagenase, whereas colon carcinoma cells cocultivated with nude mouse skin fibroblasts did not. Incubation of human colon carcinoma cells in serum-free medium containing recombinant human interferon-β (fibroblast interferon) significantly reduced gelatinase activity [73]. These *in vitro* data support the *in vivo* data that organ-specific factors can influence the invasive and metastatic properties of cancer cells.

In most patients with colon cancer, metastasis to regional lymph nodes or the liver is likely to have occurred prior to diagnosis and surgical resection of the primary tumor [74]. Thus, prognosis for patients with advanced disease with metastases to the liver and the lungs is poor. Indeed, many chemotherapeutic drugs and drug combinations have produced only marginal results [74, 75].

Since we have been interested in obtaining a better understanding of the biology of colon carcinoma metastasis, we wished to determine whether the organ microenvironment could influence the response of human colon carcinoma to systemic therapy with DXR. Highly metastatic human colon cancer KM12L4 cells previously selected for production of liver metastases in nude mice, were implanted into 3 different organ sites of nude mice: the s.c. space, the cecal wall, and the liver [76]. Tumors growing s.c. were most sensitive to DXR (Fig. 1), while tumors growing in the liver were least sensitive (Fig. 2). The differences observed *in vivo* were not evident in cultures established *in vitro*. After 1–2 weeks in culture, cells derived from untreated s.c. tumors and liver tumors were as sensitive to DXR *in vitro* as the parental KM12L4 cells [76]. These data suggest that the organ-specific differences in DXR sensitivity that we observed under *in vivo* conditions were not due to selection of different cell populations but to environmental factors that endowed tumor cells with certain properties that enhanced their resistance to systemic therapy [76].

The distribution of DXR was lowest in tumors growing s.c., followed by tumors growing in the cecum and the liver; therefore, it cannot explain the *in*

Table 1. DXR distribution in organs of nude mouse and in KM12L4 tumors

	DXR concentration (μg/g tissue)					
	Normal organs			Tumors		
Plasma[a]	0.6 ± 0.3	0.3 ± 0.1	0.1 ± 0.0	–	–	–
Cecum	4.2 ± 0.1	5.2 ± 2.0	2.6 ± 1.0	2.6 ± 1.1	2.4 ± 0.7	1.5 ± 0.2
Liver	7.3 ± 0.4	5.1 ± 0.6	3.3 ± 1.1	2.6 ± 0.4	3.4 ± 0.4	1.9 ± 0.4
Skin	2.2 ± 0.1	1.0 ± 0.0	0.9 ± 0.2	1.2 ± 0.2	1.7 ± 0.3	0.9 ± 0.1

[a] μg DXR/ml.

vivo differences in DXR sensitivity (Table 1). PKC activity was highest in samples of s.c. origin, where tumors were most sensitive to DXR, and significantly lower in samples from the liver and the cecum. Downregulation of PKC has been reported, especially in cell systems with high intrinsic PKC activity [46, 77–79]. In tumors of the colon, PKC activity has been found to be downregulated compared to adjacent mucosa [79]. We suggest that downregulation of PKC had occurred in KM12L4 cells growing in the liver or the cecum and that biliary acids may have contributed to downregulation at these organ sites [46, 80].

P-glycoprotein is a transmembrane transport protein that mediates the efflux of naturally occurring toxic products through an active transport mechanism [33–39, 80–84]. The protein is physiologically expressed in cells of a variety of human tissues including cells of the proximal tubules, the luminal surface of the colon mucosa, and the biliary canalicular surface of hepatocytes [85, 86]. Its expression in these excretory organs suggests that P-glycoprotein plays a physiological role in cell clearance of extrinsic or intrinsic toxic products. Human tumors originating from these organ sites usually exhibit high levels of P-glycoprotein or its mRNA [82, 87–91], indicating that the signal for P-glycoprotein expression can be maintained during neoplastic transformation [90]. We analyzed the expression of P-glycoprotein in KM12L4 cells harvested from tumors growing in different organs by flow cytometric analyses (Fig. 3). The data show that P-glycoprotein expression was significantly higher in cells harvested from liver and cecum tumors than in cells harvested from tumors growing s.c. and cells growing in culture. This may account at least in part for the differences observed in DXR sensitivity. It is of special interest that P-glycoprotein was elevated in organs with physiologic P-glycoprotein expression and also in organs that are physiologically exposed to biliary acids. When cells were harvested from a liver metastasis and maintained under culture conditions, expression of P-glycoprotein gradually diminished to the level of parental cultured KM12L4 cells (Fig. 4). This suggests that overexpression of P-glycoprotein in cells from tumors growing in the liver or in the cecal wall was transient, lasting only as long as they remained in the liver or cecum [76].

Functional P-glycoprotein is the product of the *mdr*1 and *mdr*3 genes. Expression of the *mdr*1 gene is associated with drug resistance. The antibody C219 recognizes a small, highly conserved epitope

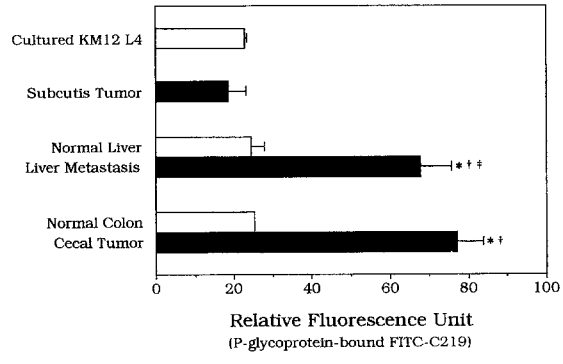

Fig. 3. Differential FITC-C219 antibody binding to P-glycoprotein of cultured human colon carcinoma cells and of those cells grown in various environments. Cells derived from tissue culture, normal organs, and tumor samples were reacted with FITC-C219. The cell-bound FITC fluorescence was analyzed by a computer and the specific expression of P-glycoprotein was presented as relative fluorescence units (RFU) normalized with the individual negative antibody fluorescence profile. The values are mean ± SEM of 3 experiments.

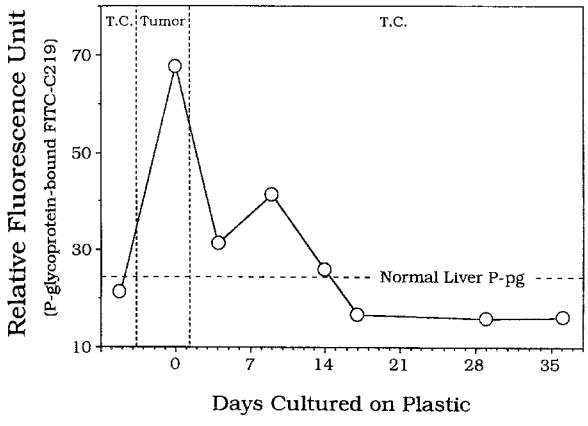

Fig. 4. Time course of FITC-C219 antibody binding of human colon carcinoma cells harvested from a liver metastasis grown in a nude mouse after reestablishing in *in vitro* culture conditions.

in the cytoplasmic domain of P-glycoprotein, accounting for high species cross-reactivity. Northern blot analyses demonstrated expression levels of both *mdr*1 and *mdr*3 that correlated with DXR sensitivity and immunohistochemical and FACS data (Fig. 5). Thus, we demonstrated that DXR sensitivity in human colon cancer cells growing in different organ sites in nude mice was modulated by the organ environments. Colon cancer cells growing in the liver and the cecum were less sensitive to DXR than cells growing s.c., perhaps because of transient overexpression of P-glycoprotein. The significance of downregulation of PKC activity is not yet known.

Murine colon carcinoma

In the next set of experiments, we implanted murine CT-26 colon carcinoma cells into different organs of syngeneic BALB/c mice [91]. We then determined whether the organ microenvironment could influence the response of murine colon carcinoma cells to systemic therapy with DXR or 5-FU. We found differences in sensitivity of murine colon cancer cells growing in the subcutis, spleen, liver, and lung to DXR (a cytotoxic agent affected by the MDR phenotype) and 5-FU (a compound unaffected by MDR), two structurally and pharmacologically distinct cytotoxic agents. The sensitivity of the CT-26 cells to DXR was highest in the s.c. environment, intermediate in the spleen and cecum, and

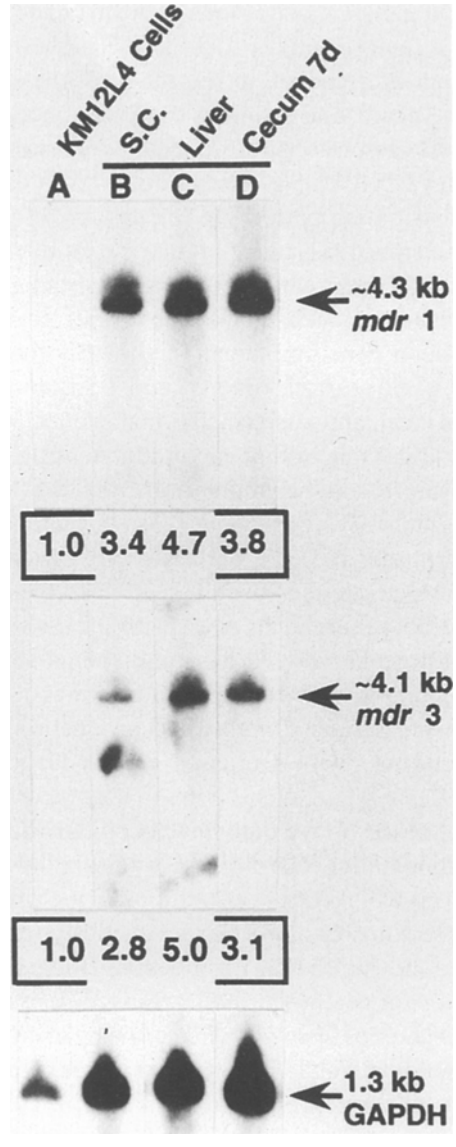

Fig. 5. Northern blot analyses of *mdr*1 and *mdr*3 expression in human colon carcinoma cells growing *in vivo* or *in vitro*. Poly (A+) mRNA (5 μg/lane) was used in all cases. The probes used are described in Materials and methods. Lane A, culture cells; lane B, subcutaneous tumor; lane C, liver tumor; lane D, cecal wall tumor grown *in vitro* 7 days. Densitometric quantitation is shown below each blot, where *mdr*1 or *mdr*3 mRNA expression was normalized to GAPDH mRNA levels, and in each case, the culture cells defined as 1.0.

lowest at metastatic sites such as the liver and lungs, whereas their sensitivity to 5-FU was highest in the lung and intermediate in the subcutis, the spleen, and the cecum, and lowest in the liver. Once again, organ-site-associated differences in drug sensitivity to either DXR or 5-FU were not associated with drug distribution patterns in the tumors.

The intrinsic resistance of tumor cells to chemotherapeutic drugs can be mediated by both genetic and epigenetic mechanisms. The former can develop through gene amplification (notably the *mdr*1 gene), gene rearrangements, and transcriptional, translational, and posttranslational events [80, 92]. Phenotypic changes that can modulate drug resistance are often associated with increased activities of drug-detoxifying enzymes [93, 94], metabolism-regulating enzymes [95], and efflux proteins [80, 92, 96]. In addition, increased levels of PKC activity have been observed in several MDR tumor cell lines [46]. Similar effects have also been associated with decreased activity of specific enzymes or influx of transmembrane proteins and production of altered enzymes with decreased affinity for a given drug [97].

Most of the above data have been derived from examining tumor cells growing in culture; however, the relevance of culture conditions to the situation *in vivo* is uncertain. We have previously reported that the mouse UV-2237 fibrosarcoma and human KM12 colon carcinoma [98] exhibit different patterns of chemosensitivity when growing in different organs of nude mice, patterns that neither correlated with nor were predicted from *in vitro* cultures. The present data extend these observations.

In some experimental systems, anthracycline antibiotics such as DXR have been shown to be more effective under hypoxic conditions, which support the formation of free radicals [20, 22]. The DXR-free radical can intercalate with DNA and promote oxidation of a variety of intracellular components [22]. In contrast, cytotoxicity mediated by 5-FU is not subject to intracellular redox regulation [59, 99]. Tumors growing s.c. may be less oxygenated than those growing in the lungs, and this may, in part, explain the sensitivity of CT-26 tumors to DXR when implanted s.c., its resistance to DXR when growing in the lung, and its sensitivity to 5-FU in the same organ.

Drug metabolism may contribute to tumor response in different organs. The catabolic inactivation of 5-FU occurs mainly in the liver, an organ with intense dihydrouracil dehydrogenase activity, which degrades 5-FU to dihydro-5-FU [100, 101]. From drug distribution analyses published previously and the present data, we concluded that the sensitivity of s.c. tumors to chemotherapy is not due to increased accumulation of these agents. Moreover, the 5-FU sensitivity of CT-26 s.c. tumors was independent of tumor size and time of initial treatment. This was not the case for DXR, in which larger s.c. tumors (6 mm) containing some necrotic zones were more sensitive to therapy than smaller s.c. tumors (0.2 to 1.5 mm). These data suggest that the sensitivity of CT-26 tumors to 5-FU and DXR did not directly correlate with the degree of vascularization.

The level of PKC activity has been directly correlated with resistance of murine UV-2237 fibrosarcoma cells to DXR, especially of those with the MDR phenotype [46]. PKC is involved in signal transduction of hormones and growth factors, and its activity can be stimulated by a variety of tumor promoters that include biliary acids [102]. Biliary acids have in turn been implicated as promoters for colon and liver cancers [46, 102]. Since both colon and hepatocellular carcinomas demonstrate high levels of resistance to many chemotherapeutic drugs, we wished to determine whether the level of PKC activity was elevated in CT-26 tumors growing in organs exposed to biliary acids, e.g., colon and liver. We found that PKC activity was low in s.c. CT-26 tumors, higher in cecal tumors, and highest in liver and spleen tumors. The high level PKC activity in the spleen was unexpected, and its significance is not clearly understood.

Organ-specific modulation of steady-state *mdr*1 gene expression in murine colon cancer cells

Most human and rodent neoplasms display heterogeneity for many properties, including sensitivity to anticancer drugs or biologicals, and many colon

cancer cells exhibit an intrinsic MDR phenotype [103–106]. The elevated expression of *mdr*1 mRNA and P-gp in CT-26 cells growing in the lung could therefore have been due to the selection of resistant variant cells. Several lines of evidence, however, suggest that the increased resistance to DXR in the CT-26 cells in lung metastases was not due to selection of resistant subpopulations. First, unlike most tumor cells selected *in vitro* for the MDR phenotype by exposure to anticancer drugs [107, 108], the CT-26 cells growing in the lung did not contain amplified *mdr*1. Second, once implanted into the subcutis of syngeneic mice, CT-26 cells from lung metastases produced tumors that were sensitive to DXR. In parallel studies, DXR-sensitive CT-26 cells from s.c. tumors became resistant to the drug when they were inoculated i.v. and grew in the lung parenchyma as metastases. Steady-state *mdr*1 mRNA levels directly correlated with the drug resistance phenotype in these experiments. Third, the increased resistance to DXR and elevated levels of *mdr*1 mRNA and P-gp were all transient in CT-26 growing in the lung. During growth in culture for > 7 days, *mdr*1 mRNA and P-gp reverted to the baseline levels of CT-26 parental cells.

The exact mechanism by which the organ environment, e.g., lung, regulates the expression of the *mdr*1 gene in CT-26 colon cancer cells is unclear. One possibility is that the organ produces organ-specific paracrine growth factors. Several lines of evidence support this possibility. First, liver regeneration results in the overexpression of MDR genes in rapidly dividing hepatocytes [109, 110], which respond to mitogenic signals, such as transforming growth factor alpha (TGF-α) [111, 112] and hepatocyte growth factor [113]. Second, growth factors found in serum, TGF-α, and platelet-derived growth factor simultaneously stimulate cell growth and activate the *mdr*1 gene promoter through the protooncogene c-*raf* kinase-related signal transduction pathway [114]. Third, blockage of the EGF-receptor-TGF-α autocrine loop with antireceptor antibodies followed by systemic administration of DXR has been shown to produce significant antitumor activity in several experimental systems [115].

In addition to organ-derived growth factors [116], the extracellular matrix can stimulate expression of the *mdr*1 gene. Hepatocytes growing *in vitro* on collagen type I demonstrate high levels of *mdr*1 gene and increased resistance to DXR, decreased DXR accumulation, and enhanced drug efflux [117]. Some physical and chemical insults may also regulate *mdr*1 gene expression [118, 119]; however, the probability that these are organ specific is low.

Modulation of *mdr*1 gene expression could occur at the transcriptional or posttranscriptional level. In regenerating rat livers, *mdr*1 expression appears to be posttranscriptionally regulated since nuclear run-on experiments showed hepatectomy had no major effect on transcription [110]. A direct demonstration of increased half-life of *mdr*1 mRNA after toxic injury to the liver or partial hepatectomy has not been shown to date [120]. Endogenous *mdr*1 RNA levels is elevated in rodent and human cells and in human KB cells transfected with a reporter construct fused to 2 kb of the *mdr*1 gene promoter after they are exposed to a variety of chemotherapeutic agents, although no direct effects of these agents on transcription were detected in nuclear run-on experiments [121, 122]. Heat shock consensus elements have been localized in the *mdr*1 promoter region, and *mdr*1 transcription is regulated by heat shock, arsenite, and cadmium in a human kidney cell line [118]. Enhanced expression of the *mdr*1 gene was found in cells cotransfected with an activated *ras* gene and a mutant *p53* gene and in cells transfected with the *mdr*1 promoter linked to a reporter gene [123]. In contrast, cells expressing wild-type *p53* exhibited specific repression of *mdr*1 mRNA [120]. These results suggest that the transcription of the *mdr*1 gene can be activated during tumor progression. Better understanding of the transcriptional regulation of rodent and human *mdr* genes requires additional information on the structure and activity of the *mdr* promoter region [120].

Conclusions

We have shown that the *in vivo* sensitivity of murine CT-26 colon carcinoma cells to DXR depends on the organ environment. The organ environment

can induce the P-gp-associated MDR phenotype in tumor cells. The expression of *mdr*1 mRNA and P-gp is transient: once removed from the environment (lung), the cells' resistance reverts to that of the sensitive parent cells. This environmental regulation of the MDR phenotype may explain many of the discrepancies between *in vivo* and *in vitro* studies designed to identify mechanisms of tumor cell resistance to chemotherapy. In any event, the model described here can be used to investigate molecular mechanisms that regulate the *in vivo* expression of the *mdr*1 gene.

Key unanswered questions

1. Can differential sensitivity to chemotherapy be demonstrated for human tumors other than colon carcinomas?
2. Is the apparent resistance of brain tumor and brain metastases to systemic chemotherapy associated with modulation of the MDR phenotype?
3. What is the role of organ-specific cytokines in the regulation of the MDR phenotype?

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