Histoculture and the immunodeficient mouse come to the cancer clinic: Rational approaches to individualizing cancer therapy and new drug evaluation (Review)

ROBERT M. HOFFMAN[1,2]

[1] Laboratory of Cancer Biology, University of California, San Diego 0609F, La Jolla, California 92093-0609;
[2] AntiCancer, Inc., 5325 Metro Street, San Diego, California 92110, USA

Received June 16, 1992; Accepted June 29, 1992

Abstract. Originating from the experiments of Alexis Carrel, tissues in culture were originally grown in three dimensions and maintained important *in vivo*-like structural and functional properties. However, in modern times, monolayer cell culture methods have become predominant despite losses of structural and functional properties of the cells. Strangeways, Fell, Leighton, Sutherland and others have designed various methods of three-dimensional culture using cellulose supports, mesh supports, collagen gel or sponge supports and floatation that allow tissues to maintain many *in vivo*-like properties such as native architecture, differentiated functions, gene regulation, invasive properties and drug sensitivities which are very different than cells in monolayer cultures. Collagen-sponge-gel-supported histoculture has been shown to support the growth and native three-dimensional architecture of both tumor and normal tissue, often for long periods of time. This method of histoculture was utilized to develop a chemosensitivity assay for individual cancer patients by assessing the effects of drug on the patients' histocultured tumor. Various end points to measure drug response have been utilized in histoculture, including [^{3}H]thymidine incorporation measured by histological autoradiography and the use of vital dyes to indicate cell viability. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide (MTT) end point was applied to the histoculture assay in an attempt to increase *in vitro-in vivo* correlation. The chemosensitivities of 16 human tumor lines were determined *in vitro* by the histoculture assay, and retrospectively correlated to their *in vivo* chemosensitivity as xenografts in nude mice. The overall correlation rate of the efficacy results of the drug-response assay to *in vivo* chemosensitivities was 89.8%, with 90.0% true-positive and 89.7% true-negative rates, 81.7% sensitivity and 94.6% specificity, thereby indicating potential clinical use for tumor histoculture with the MTT end point. The data reviewed and analyzed here thus indicate that three-dimensional culture systems offer much more realistic model systems for evaluating potential new cancer agents and individualized treatment such as predictive drug-response testing. The 'MetaMouse' model developed in our laboratory allows direct 'onplantation' of intact patient surgical cancer specimens orthotopically to athymic 'nude' mice with high-level expression of local growth on the target organ and high metastatic potential. Eight MetaMouse human cancer models are reviewed including those for the colon, bladder, lung, stomach, prostate, ovary, pancreas and head and neck. The human tumors growing and metastasizing in the mice reflect the clinical situation and should be useful for new drug evaluation and development of strategies for individual treatment. The combined technologies of histoculture and MetaMouse thus offer an integrated *in vitro-in vivo* system for preclinical evaluation of experimental and standard cancer therapy.

Contents

1. Three-dimensional histoculture and its clinical application

In 1912, Alexis Carrel took a small fragment of the heart of an eighteen-day-old chick embryo and explanted it on hypotonic plasma (1). The heart tissue grew and was passaged eighteen times over a period of 3 months. During this time, the tissue continued to beat rhythmically, thereby showing that explanted tissue could retain its normal function *in vitro* for a long period of time. Carrel understood not only the potential of tissue growth in three-dimensional culture, but also the limitations of this technique. He noted that the cultures did not increase in size and that they assumed a

Correspondence to: Prof. Robert M. Hoffman, Laboratory of Cancer Biology, University of California, San Diego 0609F, La Jolla, California 92093-0609, USA

Key words: histoculture, drug-response assay, immunodeficient mouse, orthotopic transplantation, patient-tumor specimens, metastatic models, drug evaluation

spherical shape over time. Carrel attributed these effects to nutritional factors: that is, he postulated that the cells at the periphery of the cultures proliferated because they had an ample supply of nutrients, whereas cells in the middle of the cultures necrosed because of an inadequate supply of nutrients. To circumvent this problem, Carrel started culturing tissues on a silk veil, which prevented the plasma of the culture from retracting and becoming spherical. Thus, as early as 1912, the main tenets of three-dimensional tissue culture - or 'histoculture' as it is now known (2) - were set down. Histoculture is defined in this review as culture in three dimensions that demonstrates at least some properties of tissue. Monolayer culture in this review is classified as two-dimensional culture.

By 1951, monolayer cell cultures, in which cells grew as 'sheets' on the surfaces of glass or plastic, had become the predominant culture technique. J. Leighton pointed out (3) that such monolayer cultures had rather limited use in the study of histogenesis of normal and neoplastic tissue and for pathological diagnosis. In order to allow tissues to grow in an organized three-dimensional manner, Leighton improved upon the earlier studies of Carrel by utilizing cellulose sponges that were surrounded by plasma clots in glass tubes. On each sponge, he placed 1-4 fragments of tissue, ranging in size from 1-5 mm^3, along with a single drop of chicken plasma and a drop of diluted chick embryo extract. After the plasma clotted and the tissue fragments firmly adhered to the sponge, he added a nutrient mixture and put the tubes in a roller apparatus.

Leighton grew a number of tissues, including C3HBA mouse mammary adenocarcinoma, on this cellulose sponge matrix system. The adenocarcinoma outgrowth on the sponge matrix consisted of aggregations of cells that were organized much like those in the original tumor. Structures with distinct lumina were observed and, importantly, stromal elements were associated with some of the glandular structures thereby making the tissues *in vivo*-like and allowing interactions of the different components of tissue. The stromal areas were sparse and consisted of spindle-shaped fibroblasts and a smaller number of histiocytes. Cells growing on the plasma clot without the sponge were organized in sheets and displayed no tissue-like structure. Fragments of chick embryo liver were also cultured on the sponge system, and in such cultures proliferating epithelial cells were observed to form filaments, irregular oval masses, and glandular structures. In the central part of the sponge, the interstices were filled with masses of necrotic epithelial cells. When Leighton grew hepatoma cultures on the sponges (4), he noted that, in contrast to normal liver cells, the tumor cells were loosely packed and, in areas where the plasma clots had been liquified, the cells were in direct contact with the liquid nutrient.

Leighton used natural and gelatin sponges in addition to those made of cellulose to provide physical support for the organized aggregations of cells. Cellulose sponges were digested slowly by mammary tumors studied but were found to be very brittle after infiltration with paraffin, which made it difficult to prepare histological sections. Gelatin sponges were easily sectioned but were digested rapidly by several normal tissues and tumors. Collagen-coated cellulose sponges were shown to be optimal for supporting the native architecture of tissue (5).

Table I summarizes the properties of the sponge matrix which are important for its ability to maintain the native structure of tissues in culture.

Table I. Properties of the sponge matrix that are important for the maintenance of the native structure of tissues in culture.[a]

- Has a particular spatial arrangement of trabeculae that enables cells to grow and migrate in all directions.
- Creates a large increase in surface area, which supports the growth of epithelial cells.
- Reduces the diffusion rate within cell aggregates, which may allow retention of critical factors secreted by cells.
- Permits the development of morphologic diffusion gradients that may influence tissue differentiation.
- Creates a 'wick' that may substitute for capillary circulation in allowing intercellular exchange of metabolites.

[a] For details, see reference 4.

Elegant studies by G. Heppner and coworkers have provided convincing evidence that three-dimensional cultures are more accurate models of drug resistance *in vivo* than are cell monolayers (6-8). These researchers designed an *in vitro* assay system in which cell clusters or tumor fragments were embedded in a collagen gel matrix and tumor growth was quantitated by planimetry. Cells grown under these conditions were found to be less sensitive to the drugs tested than those grown as monolayers on collagen or plastic. In some cases, the differences in sensitivity were very great; for example, with mouse mammary adenocarcinoma cell lines, the IC_{90} for doxorubicin was 100-fold greater in three-dimensional cultures than in monolayers. The drugs melphalan, methotrexate, and 5-fluorouracil caused

exponential decreases in the growth rates of monolayer cultures of mouse mammary tumor cells, but they inhibited the growth of cells in collagen gel cultures to a lesser degree, 1,000-fold less for melphalan, 300-fold less for methotrexate, and 50-fold less for 5-fluorouracil. Some histocultures in collagen continued to grow in the presence of drug concentrations that reduced survival to less than 0.1% in 'two-dimensional' cultures. If cells in collagen gel cultures were replated as monolayers, they became sensitive to the drugs. Interestingly, if the mammary tumor cells in monolayer culture were treated with melphalan and 5-fluorouracil and then transferred to the collagen gel system, the cultures became resistant to the drugs, thus indicating that drug diffusion is not the key to the greater resistance of spheroids to drugs. Thus, it is proposed that in the three-dimensional configuration, cells may assume particular shapes or attain degrees of differentiation that are important for the mechanism of drug resistance. Heppner and her colleagues point out that studies of the mechanism of resistance of three-dimensional culture may help to explain why tumors can be drug resistant *in vivo* even though monolayer cultures prepared from these tumors are drug sensitive.

R.M. Hoffman *et al* (9-13,68,69) have adapted three-dimensional histoculture on collagen sponge gels for use in predictive assays of chemotherapeutic response and evaluation of new cancer drugs. This system extends Leighton's work to actual human tumor specimens, and the system differs from Heppner's (6-8) in that the tumors are explanted on collagen sponges rather than embedding cells within collagen gels. For *in vitro* chemosensitivity testing of patients' tumors and the evaluation of potential cancer drugs, histological autoradiography to measure [^{3}H]thymidine incorporation has been used as an end point for drug response. The optical sectioning property of confocal microscopy has been used to measure fluorescent dye inclusion and exclusion to indicate cytotoxicity as another end point of drug response. The ability of tumors in histoculture to reduce tetrazolium dyes is yet another end point for drug response. Collagen-sponge-gel histocultures closely approximate the *in vivo* state in that the tissues are growing in three dimensions and contain not only the tumor cells but stromal cells such as fibroblasts and lymphocytes as well as extracellular matrix components, all maintained in native architecture. Over 25 types of human tumors and many corresponding normal tissue types have been successfully cultured in this way. The main advantages of this system are listed in Table II.

The degree of proliferation of cells within the histocultures has been shown to correlate with tumor stage and grade in the case of breast and ovarian tumors (12), and with tumor phenotype (small-cell vs. non-small-cell) in the case of lung tumors (13).

Perrapato *et al* have recently used this native-state method for drug-response analysis of urological tumors (14). They reported a high culture success ratio, longevity in culture, maintenance of primary histopathology, and reproducible chemosensitivity.

It is now agreed upon that individual tumors may consist of many types of cells, both tumor and normal, and that they may have considerably different properties including drug response (15-26). Indeed, some of the cells within a tumor may go on to dominate the tumor and produce metastases. Kerbel (27) has termed this phenomenon clonal dominance. The cells which eventually dominate a tumor and have the greatest metastatic potential, indeed, may be the most rapidly-growing cells *in vitro* as well. More directly to the point of *in vitro* drug-response assays, Murray *et al* (24) as long ago as 1954 have noted not only do drugs have different effects *in vitro* on tumors of comparable architecture from different patients but that the variation of responses to specific drugs can be seen in different areas of the same tumor. Therefore, it is important that a tumor-culture system be able to culture the multiple cell types of a tumor in their native architecture.

Table II. Advantages of native state histoculture.

- Cultures tissues in three dimensions preserving native tissue architecture.
- Detects proliferation by histological autoradiography and polarization microscopy, yielding very high resolution data, the collection of which can be semi-automated by digital pixel analysis.
- Allows determination of cell viability indicated by fluorescent vital dyes in three dimensions by confocal scanning-laser microscopy.
- Determination of tumor metabolic activity is possible by measurement of reduction of tetrazolium dyes detected by spectrophotometry or pixel analysis.
- Offers a very high rate of evaluation of drug response for all tumor types - approximately, 80% or better.
- Allows simultaneous testing of multiple agents.
- Uses relatively small amounts of tissue - as small as 100 mg.
- Produces a low frequency of false negatives and false positives, as assessed by correlative drug response studies *in vitro* and *in vivo*.
- Allows normal and tumor tissue to be cultured in parallel, so that the tumor specificity of anti-neoplastic agents can be assessed.
- Allows assay of a wide range of antineoplastic agents (e.g., cytotoxics, biological response modifiers, activated immune cells).

The proper *in vivo*-like growth of tumors *in vitro* is necessary for the design of an optimal drug-response assay but is not sufficient. The parameters or 'end points' to measure drug response must be well chosen. The measurement of thymidine incorporation, for example, into cell nuclei allows the determination of the effects of drugs on DNA synthesis.

Histological autoradiography allows the measurement of a morphological end point in that the cell types observed incorporating [^{3}H]thymidine by grain formation in the autoradiograms can be simultaneously identified morphologically in sponge-matrix culture. The vast majority of the proliferating cells are usually the tumor cells. This allows one the possibility to use the much simpler procedure of scintillation counting to measure [^{3}H]thymidine uptake. The key aspect of using [^{3}H]thymidine uptake measurement by scintillation counting as an end point to measure drug response is to have another parameter to normalize thymidine uptake to, possibly a non-metabolized radio-biochemical taken up by all cells.

However, the end point of thymidine uptake does not allow the measurement of the effects of drugs in the G_0 phase of the cell cycle. This can be overcome by using cell death as an end point with the use of vital-dye-inclusion and exclusion. For example, dyes that enter into cells and fluoresce only if the membrane is no longer intact report dead cells, and dyes that enter and fluoresce only if the cells have an active enzyme system that cleaves them to the fluorescence state report live cells.

Weisenthal and Lippman (28) point out key critical technical problems in using dye-exclusion and -inclusion as end points. Sufficient time must elapse following drug treatment for lethally-damaged cells to lose their membrane integrity. Therefore, the proper time of measurement of dye uptake after drug treatment is critical. Pavlic (29) in this light has demonstrated that the exposure of tumor cells even to very high concentration of anticancer agents can inhibit *in vitro* proliferation while esterase activity needed to activate fluorescent carboxy-fluorocein-based dyes or the ability to exclude dyes such as propidium iodide are persistently retained demonstrating the potential of recovery after drug exposure. It is only after a progressive decline of cell proliferation on days 4 and 7 following drug exposure that proliferative recovery did not occur and the cells, indeed, lost viability. Pavlic et al argued that potential proliferative recovery of cancer cells may explain the excessive false-positives in chemosensitive assays based on proliferative end points.

In a recent study (30), the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide (MTT) end point was applied to the histoculture assay in an attempt to increase *in vitro-in vivo* correlation. The chemosensitivities of 16 human tumor lines were determined *in vitro* by the histoculture assay, and retrospectively correlated to their *in vivo* chemosensitivity as xenografts in nude mice. The overall correlation rate of the efficacy results of the drug-response assay to *in vivo* chemosensitivities was 89.8%, with 90.0% true-positive and 89.7% true-negative rates, 81.7% sensitivity and 94.6% specificity, thereby indicating potential clinical use for tumor histoculture with the MTT end point.

Glucose consumption has been also used as an end point in histoculture and this continuously measurable, non-invasive end point has demonstrated that tumors may recover from apparently lethal drug effects after long periods (67).

Slocum *et al* (31) have demonstrated that a number of human tumor types obtained at biopsy and initiated in the native-state histoculture sponge-matrix system can be serially passaged from one gel raft to another for periods exceeding one-and-one-half years with maintenance of at least some tissue architecture. The serially-passaged histocultures appear to take on some of the properties of established cell lines, but in this case in tissue form without *in vivo* passage. These serially-passagable histocultures have been termed histolines. These include sarcomas, colon carcinoma and melanoma thus far.

Another key aspect now possible with the native-state sponge-matrix histoculture system is to be able to study the specific nutritional requirements of tumor tissue in comparison with normal tissue. For example, in monolayer culture, cell lines derived from tumors have an absolute dependence on preformed methionine in the culture medium, whereas normal cell types can survive and grow in the presence of homocysteine in place of methionine in the culture medium used (32,33). Nutritional-requirement studies made on surgical specimens in sponge-matrix histoculture of human tumor and normal tissue indicate it is also possible to selectively starve tumor tissue by depriving the tumors of methionine (Guo H-Y, Herrera H and Hoffman RM, unpublished data). Methionine-dependent tumors, starved for methionine *in vitro*, become selectively synchronously arrested and thereby much more susceptible to cell cycle-specific drugs (34). It is important to apply this approach *in vivo*.

2. Metastasizing models of human cancer in immunodeficient mice (MetaMouse) and their clinical application

In our laboratories, histologically-intact-human colon cancer specimens directly derived surgically from patients were onplanted orthotopically to the corresponding organs in immunodeficient mice for eight different-major human cancer types. We have achieved extensive orthotopic growth in fifteen of twenty-three cases of patient colon tumors with subsequent regional, lymph-node, and liver metastasis, depending on the case. Similar results were found with onplantation of intact tissue from a human colon cancer xenograft line, including liver metastasis. Thus, a patient-like model for human colon cancer has been developed that can be used for research into the biology of colon cancer metastases, the potential prediction of clinical course and drug response of the disease for individual patients, and the testing of new modalities of treatment (35,36).

With regard to bladder carcinomas, Ahlering *et al* (37) found that 2 human bladder transitional cell carcinoma lines, when injected transurethrally into the urinary bladders of athymic nude mice, invaded the mouse bladder and metastasized to the lung. Subcutaneous inoculation of these cell lines allowed tumor growth, but very little local invasion and no metastases. Recently, Theodorescu *et al* (38) confirmed the results of Ahlering with respect to the RT-4 human bladder carcinoma cell line. Theodorescu *et al* found, however, when a mutated H-*ras* oncogene was transfected

into RT-4 such that over-expression of this gene occurred in the selected cell line RT-4-mr-10, the cell line became more invasive after transurethral inoculation. Areas of invasion of transitional cell carcinoma deep into the muscularis propria of the bladder occurred which in some instances extended into the surrounding adipose tissue and vascular spaces. However, no continuous or metastatic spread of RT-4-mr-10 occurred. These findings are in stark contrast with the effects of subcutaneous injection of these cell lines which showed no evidence of tissue invasion (38).

In our laboratories, the *ras*-transfected human bladder RT-4 carcinoma tissue cell line RT-4-mr-10 described above was orthotopically onplanted as histologically-intact tissue to the nude mouse bladder. Extensive invasive orthotopic growth and local invasion occurred as well as multi-organ metastases observed in the liver, pancreas, spleen, lung, ovary, kidney, ureter and lymph nodes. The results for the onplanted RT-4-mr-10 are in striking contrast to the experiments described above where RT-4-mr-10 was injected transurethrally as disaggregated cells where only local invasion and no distant metastasis were observed. These results further indicate the potential of the intact-tissue onplant MetaMouse model of orthotopic transplantation to allow full expression of metastatic capacity of human cancer in the nude mouse (39,40). We have recently demonstrated that the RT-4 parental bladder tumor line is highly metastatic when onplanted orthotopically as histologically-intact tissue, thereby showing that the *ras*-gene had no effect (41).

Vezeridis *et al* (42) reported that the fast-growing variant of the human pancreatic carcinoma COLO 357, when injected as disaggregated cells into the spleen of the nude mice, resulted in metastases to the liver and lungs of the animal. The authors stated, however, that this study bypasses invasion and generates seeding and colonization rather than metastases. A subsequent study was carried out (43) using the COLO 357 and L 3.3 human pancreatic tumors to compare orthotopic transplantation with subcutaneous inoculation. These authors took tumors that were subcutaneously grown and harvested and sectioned them into 2 x 2 mm pieces. Xenografts were first attached to the exteriorized pancreas. The pancreas was then wrapped around the xenograft to cover it completely. The edges of the fatty tissue surrounding the pancreas were sutured such that the xenograft would remain covered upon the return of the pancreas to the peritoneal cavity. It was found that the majority of the animals grew tumors at the orthotopic site of transplantation. Metastases occurred in the liver, lung, regional lymph nodes and distant lymph nodes. The authors felt that by using tumor pieces as xenografts rather than injecting tumor cells into the pancreas, the probability of injecting tumor cells into the circulation with subsequent seeding and colonization was eliminated. They emphasized that their model was similar to the human situation of pancreatic cancer where retro-peritoneal nodes, liver and the lungs become involved.

Marincola *et al* (44), also orthotopically implanted human pancreatic-cancer cell lines, in their case in the duodenal lobe of the pancreas for comparison with heterotopic implantation at the hepatic and subcutaneous sites. Intrapancreatic tumor growth was occasionally associated with liver metastases in the animals that were killed after 28 days: 17.8% in young animals and 22.2% in adult animals. However, after more than 45 days of tumor growth, the incidence of hepatic metastases increased to 57.1%. Direct extension of the tumor into surrounding tissues was observed frequently with involvement of the duodenum in 84% of the mice, kidneys in 31%, and other intra-abdominal organs in 44%. Subcutaneously-growing tumors did not give rise to detectable metastases.

In our laboratories, we have utilized the MetaMouse technique to onplant histologically-intact human pancreatic tumor tissue to the pancreas of the nude mouse and have achieved tumor growth in six out of six patient cases (45). Extensive local growth occurred in all cases with regional extension and frequent metastases to lymph nodes and visceral organs (45).

With regard to head and neck tumors, Dinesman *et al* (46) implanted 42 nude mice with laryngeal squamous cell carcinoma in the form of cell lines to the floor of the mouth. Pulmonary metastases were noted in 44%, bone invasion in 80%, angioinvasion in 76% and soft tissue invasion in 96% of the animals, thereby mimicking the clinical state. In the head and neck study, lymph node metastasis was seen in only 2 of 42 animals. In comparison, the subcutaneous model of transplantation for head and neck tumors has not resulted in metastases of tumors that did eventually take, which was at a low rate. For example, Braakhuis *et al* (47) implanted 130 head and neck carcinomas in subcutaneous tissues of nude mice with a 26% take rate with no observed metastases.

Our laboratory has utilized tumor material directly from surgery from human head and neck cancer patients, including metastatic tongue and laryngeal tumors and planted them as histologically-intact tissue into the muscles of the floor of the mouth including the mylohyoid muscle as further examples of the MetaMouse technique. We have observed subsequent invasion into the structures of the head (Fu X and Hoffman RM, unpublished data). When the same tumor tissue was implanted subcutaneously, even in the neck area, extensive tumor growth occurred without subsequent invasion.

The human gastric cancer cell line G/F was implanted either subcutaneously or into the stomach wall of nude mice (48). The G/F tumor implanted in the stomach wall showed a slower growth rate than when the tumor was implanted subcutaneously. Importantly, the tumor implanted in the stomach wall grew and invaded the surrounding tissues and metastasized to the regional lymph nodes and distant organs such as the lung and the liver in 27 out of 43 mice. In contrast, the tumors growing subcutaneously were highly encapsulated and metastasis to other organs was not observed. Thus, the stomach wall provided a superior environment for the G/F gastric cancer to express its metastatic properties.

In our study, human-patient stomach tumors or human stomach tumors previously grown subcutaneously were onplanted histologically-intact orthotopically to the stomach wall. Subsequently, extensive local growth occurred as well as metastasis to the liver and pancreas, further demonstrating the potential of the MetaMouse model. As with the bladder and colon tumors, onplantation of histologically-intact tissue resulted in much higher

metastatic rates than orthotopic injection of cell suspensions (Furukawa T, Kubota T and Hoffman RM, unpublished data).

Recent studies have demonstrated that inoculation of human lung tumor cell lines intrathoracically or intrabronchially into nude mice (49,50) results in orthotopic growth.

The MetaMouse approach utilizing histologically-intact tumor tissue was applied to lung tumor implantation in the left lung by a thoracotomy procedure. Results thus far (51) indicate that this method not only allows extensive local growth in nude and scid mice, but allows development of regional and distant metastases as described above.

When poorly-differentiated large-cell-squamous-cell patient tumor #2268, was transplanted orthotopically to the left lung as histologically-intact tissue directly from surgery, 5 out of 5 mice produced locally-growing tumors. Opposite-lung metastases occurred, as well as lymph node metastases. When grown subcutaneously, this tumor grew locally but no metastases were found (51).

When the human small-cell lung carcinoma cell line Lu-24 was transplanted histologically intact into the left lung of nude mice via thoracotomy after harvesting of subcutaneously-growing tissue from nude mice, 5 out of 5 mice produced locally-growing tumors averaging 10 mm in diameter within 24 days. All 5 mice produced regional metastases including tumor invasion of the mediastinum, the chest wall and pericardium, and distant metastases including the right lung, esophagus, diaphragm, parietal pleura and lymph nodes. These 5 mice were implanted with only one 1.5 mm^3 piece of tissue. Three severe-combined-immunodeficient (SCID) mice were also implanted orthotopically with histologically-intact Lu-24 tissue via thoracotomy. All 3 animals produced locally-growing tumors averaging 7.5 mm in diameter within 17 days. All three scid mice also developed regional metastases including the mediastinum, left chest wall and pericardium and distant metastases including the opposite lung, lymph nodes, parietal pleura and diaphragm. The time when symptoms could be observed in the nude mice after transplantation of Lu-24 via thoracotomy was 24 days, as mentioned above, but in the SCIDs, only 17 days, with the tumor seemingly growing and metastasizing more rapidly in the SCIDs (51).

Similar results were found after orthotopically transplanting histologically-intact tissue of human-small-cell lung carcinomas Lu-130 and H-69 with very large local growth and metastases to the opposite lung and distant lymph nodes resulting (70), and in the case of Lu-130, metastasis to the brain. These results contrast with the orthotopic injection of small-cell carcinoma in nude rats resulting in poor local growth and no metastases (52,53).

Orthotopic transplantation of intact tissue of the human lung adenocarcinoma A549 has resulted in local growth and metastases to the opposite lung and lymph nodes (71).

A number of cell lines from human prostatic carcinoma that grow in athymic nude mice including the LnCap which is androgen dependent (54), and the androgen-independent cell lines Du145 (55) and PC-3 (56,57) have also been isolated. The usual mode of *in vivo* growth of the human prostate carcinoma lines has been after subcutaneous transplantation. The use of extracellular matrix proteins such as Matrigel seems to enhance the take rate of subcutaneously-implanted tumors (58). However, intra-spleenic injection (59) has also been used which may result in more metastatic activity. The PC-3 line has been injected into the tail vein of nude mice while the inferior vena cava was occluded which allowed tumor growth in the lumbar vertebrae, pelvis and femurs (60). When PC-3 cells were injected into the peritoneal cavity, intraabdominal growth resulted (61). When injected into the spleen, liver metastases resulted and when injected into the seminal vesicles, large tumors developed there (61).

The human hormone-independent prostate-cancer line Du145 was grown subcutaneously in nude mice in our laboratory. The subcutaneously-grown tumor was then resected and transplanted into nude mice orthotopically using histologically-intact tissue. Three mice were analyzed on day-71, -80, and -90 respectively, after transplantation. Tumor growth was found in all three mice. The size of the primary tumor growth ranged from 14 x 17 to 15 to 18 mm^3. The tumor appeared to be invasive and metastatic regionally. In two mice, the tumor invaded the lamina propria of the mouse urinary bladder. The local large tumor growth on the prostate of the mice caused urinary obstruction and in one mouse hydronephrosis was observed. No distal organ metastases were observed. When the human prostate carcinoma line was implanted orthotopically in nude mice as intact tissue harvested from subcutaneously-grown tumor in the nude mouse, tumors grew locally, invaded the muscularis of the bladder, kidney, inguinal, iliac and mediastinal lymph nodes. Thus, we have constructed the first orthotopic transplant model for prostate carcinoma (Fu H. and Hoffman R.M., unpublished data). The model should prove important for the development of new therapeutics and diagnostics for prostate carcinoma.

Three cases of human ovarian cancer were transplanted orthotopically into the ovarian capsule into nude mice in our laboratory. In three cases, we observed three completely different patterns of tumor growth. In the first case with patient specimen #1943, a very large encapsulated tumor, measuring 33 x 23 mm with watery fluid developed. No rupture or intraperitoneal seeding was observed. This tumor grew with a cystadenocarcinoma growth pattern. During autopsy, a very small metastatic nodule on the lung of the mouse was observed. The second case with patient specimen #2443, extensive primary tumor growth was observed. Extensive seeding on the colon and parietal peritoneum of the nude mouse was also found. In the third case, with patient specimen #2429, relatively small primary tumor growth was found, but extensive distant metastases were seen that seemed subcutaneous. These metastases also involved most of the mouse mammary glands, producing large tumor nodules in both axillary and inguinal areas.

The nude-mouse models of human ovarian carcinoma described above, therefore, have the following characteristics: (i) They can be constructed directly from patient tumor specimens. (ii) The tumors grow locally in the ovary. (iii) The tumors can metastasize to the lung, can seed and grow in the peritoneal wall and can involve critical organs such as the colon, all of which reflects the clinical

situation. In one case there was also extensive distant subcutaneous growth which can also occur in patients.

The approach to the construction of models of human ovarian cancer described here should be of eventual use clinically.

Regarding why orthotopically-growing tumors metastasize to a much greater extent than ectopically-growing tumors, Reid and Zvibel (62) indicated that investigators have identified a number of parameters: (i) anatomy, which determines the local microenvironment including the location of an available capillary bed; (ii) formation of tumor emboli; (iii) molecules that specify attachment of tumor cells to particular cells or to extracellular matrix molecules that are tissue specific; (iv) local growth factors; and (v) local matrix chemistries.

Colon tumor cells implanted intracecally grew and produced metastases and also produced higher levels of heparanase and 92-kd and 64-kd species of type IV collagenase than the same tumors planted subcutaneously which did not metastasize (63). Nakajima *et al* (63) speculate that these enzymes are critical for the enhanced metastatic capability of the orthotopically-implanted tumor.

Leighton (64) postulated that fibroblasts can orient and mediate the directionality of cell spread in metastases. Thus, the stromal elements at the orthotopic site such as fibroblasts might be more interactive with the organ-specific tumor epithelium with regard to directing cellular invasion, than at ectopic sites.

3. New directions

A mouse deficient in T-cells as well as B-cells has recently been isolated. This mouse has been termed the 'SCID' (severe combined immunodeficiency) mouse (65). Recent studies have indicated that the SCID mouse can be engrafted with human T- and B-cells as well as with human tumors (66). Thus, possibilities have opened up to put in a single animal both the patient tumor and the patient's immune system. We have termed this mouse 'SuperMouse'. These animals should allow the study of the effects of immunoenhancers and biological response modifiers in the treatment of human cancer, as well as the effects of standard cytotoxic drugs. Thus, a new era should be opening where cancer patients should be able to have rodent models established for their tumors as standard practice. Such models should enhance the identification of effective new therapies.

References

1. Carrel A: On the permanent life of tissues outside the organism. J Exp Med 15: 516-528, 1912.
2. Sherwin RP, Richters A, Yellin AE and Donovan AJ: Histoculture of human breast cancers. J Surg Oncol 13: 9-20, 1980.
3. Leighton J: A sponge matrix method for tissue culture. Formation of organized aggregates of cells *in vitro*. J Natl Cancer Inst 12: 545-561, 1951.
4. Leighton J: The growth patterns of some transplantable animal tumors in sponge matrix tissue culture. Natl Cancer Inst 15: 275-293, 1954.
5 Leighton J, Justh G, Esper M and Kronenthal R: Collagen-coated cellulose sponge: three-dimensional matrix for tissue culture of Walker tumor 256. Science 155: 1259-1261, 1967.
6. Lawler EM, FR Miller and GH Heppner: Significance of three-dimensional growth patterns of mammary tissues in collagen gels. *In Vitro* 19: 600-610, 1983.
7. Miller BE, Miller FR and Heppner GH: Assessing tumor drug sensitivity by a new *in vitro* assay which preserves tumor heterogeneity and subpopulation interactions. J Cell Physiol Suppl 3: 105-116, 1984.
8. Miller BE, Miller FR and Heppner GH: Factors affecting growth and drug sensitivity of mouse mammary tumor lines in collagen gel cultures. Cancer Res 45: 4200-4205, 1985.
9. Freeman AE and Hoffman RM: *In vivo*-like growth of human tumors *in vitro*. Proc Natl Acad Sci 83: 2694-2698, 1986.
10. Hoffman, RM, Connors KM, Meerson-Monosov AZ, Herrera H and Price JH: A general native-state method for determination of proliferation capacity of human normal and tumor tissues *in vitro*. Proc Natl Acad Sci 86: 2013-2017, 1989.
11. Vescio RA, Redfern CH, Nelson TJ, Ugoretz S, Stern PH and Hoffman RM: *In vivo*-like drug responses of human tumors growing in three-dimensional, gel-supported, primary culture. Proc Natl Acad Sci 84: 5029-5033, 1987.
12. Vescio RA, Connors KM, Youngkin T, Bordin GM, Robb JA, Umbreit JN and Hoffman RM: Cancer biology for individualized therapy: Correlation of growth fraction index in native-state culture with tumor grade and stage. Proc Natl Acad Sci, 691-695, 1990.
13. Vescio RA, Connors KM, Bordin GM, Robb JA, Youngkin T, Umbreit JN and Hoffman RM: The distinction of small cell and non-small cell lung cancer by growth in native-state histoculture. Cancer Res 50: 6095-6099, 1990.
14. Perrapato SD, Slocum HK, Huben RP, Ghosh R and Rustum Y:Assessment of human genitourinary tumors and chemosensitivity testing in 3-dimensional collagen gel culture. J Urol 143: 1041-1045, 1990.
15. Leighton J: In: Biological Interactions of Normal and Neoplastic Growth. Brennan MJ and Simpson EL (eds). Little Brown and Company, Boston. pp681-702, 1962.
16. Heppner GH, Dexter DL, DeNucci T, Miller FR, Calabresi P: Heterogeneity in drug sensitivity among tumor cell population of a single mammary tumor. Cancer Res 38: 3758-3763, 1978.
17. Heppner GH and Miller BE: Tumor heterogeneity: Biological implications and therapeutic consequences. Cancer Metastasis Reviews 2: 5023, 1983.
18. Heppner GH: Tumor heterogeneity. Cancer Res 44: 2259-2265, 1989.
19. Heppner GH: Tumor cell societies. J Natl Cancer Inst 81: 648-649, 1989.
20. Barranco SC, Ho DHW, Drewinko B, Romsdahl MM, Humphrey RM: Differential sensitivity of human melanoma cells grown *in vitro* to arabinosylcytosine. Cancer Res 32: 2733-2736, 1972.
21. Hakansson L and Trop C: Cell clones with different sensitivity to cytostatic drug in methylcholanthrene induced mouse sarcoma. Acta Pathol Microbiol Scand Sect D 82: 41-47, 1974.
22. Isuruo T and Fidler IJ: Differences in drug sensitivity among tumor cells from parental tumors, selected variants and spontaneous metastases. Cancer Res 41: 3058-3064, 1981.
23. Murray MR and Stout AP: The classification and diagnosis of human tumors by tissue culture methods. Texas Rep and Biol Med 12: 898-915, 1954.
24. Murray R, Peterson ER, Hirschberg E, Pool J: Metabolic and chemotherapeutic investigation of human glioblastoma *in vitro*. Ann NY Acad Sci 58: 1147-1171, 1954.
25. Owens AH, Coffey DH, Baylin SB (eds) Tumor Cell heterogeneity: origins and implications. Bristol-Myers Cancer Symposium. Vol 4, Acad Press NY, 1982.
26. Yung W, Shapiro J, Shapiro W: Heterogenious chemo-sensitivities of subpopulations of human glioma cells in culture. Cancer Res 42: 992-998, 1982.
27. Kerbel RS: Growth dominance of the metastatic cancer cell: Cellular and molecular aspects. Advances in Cancer Res 55: 87-132, 1990.
28. Weisenthal LM and Lippman ME: Clonogenic and non-clonogenic *in vitro* chemosensitivity assays. Cancer Treat Rep 69: 615-632, 1985.
29. Pavlik EJ, Flanigan RC, Van Nagell JR, Hanson MB, Donaldson ES, Keaton K, Bartmas J, Kenady D: Esterase activity exclusion of propidium iodine and proliferation in tumor cells exposed to anticancer agents: Phenomena relevant to chemosensitivity determinations. Cancer Inv 3: 413-426, 1985.

30. Furukawa T, Kubota T, Watanabe M, Takahara T, Yamaguchi H, Takeuchi T, Kase S, Kodaira S, Ishibiki K, Kitajima M and Hoffman RM: High *in vitro-in vivo* correlation of drug response using sponge-gel-supported three-dimensional histoculture and the MTT end point. Int J Cancer 51: 489-498, 1992.
31. Slocum H, Toth K, Li L, Chang S-G, Hoffman RM and Rustum Y: Long-term Passage of Human Tissues *In Vitro* as Three-Dimensional Histolines. In Vitro Develop Biol (In Press)
32. Hoffman RM: Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis: a review and synthesis. Biochim et Biophys Acta, Reviews on Cancer 738: 49-87, 1984.
33. Hoffman RM: Unbalanced transmethylation and the perturbation of the differentiated state leading to cancer. BioEssays 12: 163-166, 1990.
34. Stern PH and Hoffman RM: Enhanced *in vitro* selective toxicity of chemotherapeutic agents for human cancer based on a metabolic defect. J Natl Cancer Inst 76: 629-639, 1986.
35. Fu X, Besterman JM, Monosov A and Hoffman RM: Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically-intact patient specimens. Proc Natl Acad Sci USA 88: 9345-9349, 1991.
36. Fu X, Herrera H, Kubota T and Hoffman RM: Extensive liver metastasis from human colon cancer in nude and scid mice after orthotopic onplantation of histologically-intact human colon carcinoma tissue. Anticancer Res (In Press).
37. Ahlering T, Dubeau L and Jones PA: A new *in vivo* model to study invasion and metastasis of human bladder carcinoma. Cancer Res 47: 6660-6665, 1987.
38. Theodorescu D, Cornil I, Fernandez B and Kerbel R: Overexpression of normal and mutated forms of H-*ras* induces orthotopic bladder invasion in a human transitional cell carcinoma. Proc Natl Acad Sci 87: 9047-9051, 1990.
39. Fu X, Theodorescu D, Kerbel R and Hoffman RM: Extensive multi-organ metastasis in nude mouse of orthotopically-planted histologically-intact human bladder carcinoma cell line tissue. Proc Amer Assoc Cancer Res 32: 71, 1991.
40. Fu X, Theodorescu D, Kerbel RS and Hoffman RM: Extensive multi-organ metastasis following orthotopic onplantation of histologically-intact human bladder carcinoma tissue in nude mice. Int J Cancer 49: 938-939, 1991.
41. Fu X and Hoffman RM: Human RT-4 bladder carcinoma is highly metastatic in nude mice and comparable to *ras*H-transformed RT-4 when orthotopically onplanted as histologically-intact tissue. Int J Cancer 51: 989-991, 1992.
42. Vezeridis MP, Turner MP, Kajiji S, Yankee R and Meitner P: Metastasis of a human pancreatic cancer (HPC) to liver in nude mice. Proc Amer Assoc Cancer Res 26: 53, 1985.
43. Vezeridis M, Doremus C, Tibbetts L, Tzanakakis G and Jackson B: Invasion and metastasis following orthotopic transplantation of human pancreatic cancer in the nude mouse. J of Surg Onc 40: 261-265, 1989.
44. Marincola F, Drucker BJ, Siao D, Hough K and Holder WD Jr: The nude mouse as a model for the study of human pancreatic cancer. J of Surg Res 47: 520-529, 1989.
45. Fu X, Guadagni F and Hoffman RM: A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically from histologically-intact patient specimens. Proc Natl Acad Sci USA, 89: 5645-5649, 1992.
46. Dinesman A, Haughey B, Gates G, Aufdemorte T and Von Hoff D: Development of a new *in vivo* model for head and neck cancer. Otolaryongol Head Neck Surg 103: 766-774, 1990.
47. Braakhuis BJM and Snow GB: Activity of conventional drug in head and neck cancer xenografts. In: Human Tumor Xenografts in Anticancer Drug Development. Winograd B, Peckham JJ, Pinedo HM (eds.) Springer-Verlag, Germany, 1988.
48. Yamashita T: Manifestation of metastatic potential in human gastric cancer implanted into the stomach wall of nude mice. Jpn J Cancer Res 79: 945-951, 1988.
49. McLemore T, Liu M, Blacker P, Gregg M, Alley M, Abbott B, Shoemaker R, Bohlman M, Litterst C, Hubbard W, Brennan R, McMahon J, Fine P, Eggleston J, Mayo J and Boyd MR: Novel intrapulmonary model for orthotopic propagation of human lung cancers in athymic nude mice. Cancer Res 47: 5132-5140, 1987.
50. McLemore T, Eggleston J, Shoemaker R, Abbott B, Bohlman M, Liu M, Fine D, Mayo J and Boyd M: Comparison of intrapulmonary, percutaneous intrathoracic, and subcutaneous models for the propagation of human pulmonary and nonpulmonary cancer cell lines in athymic nude mice. Cancer Res 48: 2880-2886, 1988.
51. Wang X, Fu X and Hoffman RM: A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice. Int J Cancer 51: 992-995, 1992.
52. Howard R, Chu H, Zeligman B *et al:* Irridiated nude rat model for orthotopic human lung cancers. Cancer Res 51: 3274-3280; 1991.
53. Muluin O, Howard R, Mitchell D *et al:* Secondary screening system for preclinical testing of human lung cancer therapies. J Natl Cancer Inst 84: 31-37, 1992.
54. Horoszewicz J, Leonge S, Kawinski E *et al:* LNCaP model of human prostatic carcinoma. Cancer Res 43: 1809-1811, 1983.
55. Stone KR, Mickey D, Wunderli H, Mickey G and Paulson D: Isolation of a human prostate carcinoma cell line (DU 145). Int J Cancer 21: 274-281, 1978.
56. Kaighn M, Narayan K, Ohnuki Y, Lechner J and Jones LW: Establisment and characterization of a human prostatic carcinoma cell line (PC-3). Invest Urol 17: 16-23, 1975.
57. Kozlowski J, McEvan L, Keer H *et al:* In: Tumor Progression and Metastasis. Fidler IJ and Nicholson G (eds.) pp189-231, New York, Alan R Liss, 1988.
58. Pretlow T, Delmoro C, Dilley G, Spudagora C and Pretlow T: Transplantation of human prostatic carcinoma into nude mice in matrigel. Cancer Res 51, 3814-3817, 1991.
59. Sherwood E, Ford J, Lee L, Kozlowski J: Therapeutic efficacy of recombinant tumor necrosis factor alpha in an experimental model of human prostatic carcinoma. J Biol Res Mod 9: 44-52, 1990.
60. Shervin D, Kukreja S, Ghosh L and Lad T: Development of skeletal metastasis by human prostate cancer in athymic nude mice. Clin Expl Metastasis 6: 401-409, 1988.
61. Shervin D, Gorny K and Kukreja S: Patterns of metastasis by the human prostate cancer cell line PC-3 in athymic nude mice. The Prostate 15: 187-194, 1989.
62. Reid LM and Zvibel I: Production of matrix-degrading enzymes determined by tumor implantation site. J Natl Cancer Inst 82: 1866, 1990.
63. Nakajima M, Morikawa K, Fabra A, Bucana C and Fidler IJ: Influence of organ environment on extracellular matrix degradative activity and metastasis of human colon carcinoma cells. J Natl Cancer Inst 82: 1890-1898, 1990.
64. Leighton J: Spread of Human Cancer. Academic Press, New York, 1967.
65. Mosier D, Gulizia R, Baird S, Wilson D, Spector D and Spector S: Human immunodeficiency virus injection of human-PBL-SCID mice. Science 251: 791-794, 1991.
66. Bosma M, Phillips R and Schuler W: The Scid Mouse. In: Current Topics in Microbiology and Immunology. Springer Verlag, Berlin, 1989.
67. Chang S-G, Slocum H, Toth K, Hoffman RM, Perrapato SD, Huben RP and Rustum S: Glucose consumption of human tumors in primary histoculture on sponge gels as a continuously-measurable end point of drug response: New insight of the ability of tumors to recover from drug treatment. In Vitro Develop Biol (In Press).
68. Hoffman RM: Three-dimensional histoculture: Origins and applications in cancer research. Cancer Cells 3: 86-92, 1991.
69. Hoffman RM: *In vitro* sensitivity assays in cancer: A review, analysis and prognosis. J Clin Lab Anal 5: 133-143, 1991.
70. Wang X, Fu X and Hoffman RM: A new patient-like metastatic model of human small-cell lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice. Anticancer Res (In Press).
71. Wang X, Fu X and Hoffman RM: A patient-like metastasizing model of human lung adenocarcinoma constructed via thoracotomy in nude mice. Anticancer Res (In Press).