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Transplantation of Human Prostatic Carcinoma into Nude Mice in Matrigel

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Abstract

Previous successful transplantation of human primary prostatic carcinomas into nude mice has been described as "close to zero." When injected in Matrigel instead of culture medium, 25,000-fold fewer cells of the PC-3 human prostatic carcinoma cell line were required for the growth of tumors in nude mice during a 3-month period of observation; similar enhancement was observed with two other human prostatic carcinoma cell lines. Six of ten primary human prostatic carcinomas were transplanted successfully into nude mice when Matrigel was used as the vehicle.

Introduction

Prostatic carcinoma has recently become the most commonly diagnosed invasive cancer and the second most common cause of deaths from cancer among males in the United States (1). There are a few reports of the establishment of cell lines from prostatic carcinoma (2-6) and a report of the transformation of neonatal prostatic epithelial cells (7). There are no reported methods that permit the long-term growth of most human prostatic carcinomas either in vitro or in vivo. A review (8) of the efforts of many laboratories to transplant human prostatic carcinomas into nude mice resulted in the conclusion that "The incidence of 'take' of prostate cancer has been close to zero." In a recent symposium, Kleinman et al. (9) reported that an "enhanced tumorigenic potential" was observed when Matrigel was used as the vehicle for the injection into nude mice of several kinds of cells including 3T3 cells, MCF7 human mammary carcinoma cells, small cell lung carcinoma cells, and "fresh isolates of human renal tumor cells." A few months after Dr. Kleinman's report, the same group (10) reported detailed studies of small cell carcinoma of the lung that (a) were consistent with the earlier report and (b) extended the earlier study by showing that the effects of Matrigel were partially abrogated by the addition to the Matrigel and cells to be injected of a synthetic peptide from the B1 chain of laminin, one of the components of Matrigel. We now report that the use of Matrigel as the vehicle for injection of human prostatic carcinomas into nude mice enhances the growth of the injected carcinomas.

Materials and Methods

The three publicly available human prostatic carcinoma cell lines, PC-3, DU 145, and LNCaP, were obtained from the American Type Culture Collection (Rockville, MD) and were cultured under the conditions recommended by them. Human prostatic carcinomas were obtained from the branch of the National Cancer Institute National Human Tissue Network, located at Case Western Reserve University. Slices of carcinoma, 1-2 mm thick, that were used were contiguous to similar slices that were used for cryostat sections to verify the nature of the tissue transplanted. Tissues for transplantation were minced with scalpels to fragments that measured no more than 1 mm in maximum dimension. Approximately 0.2-0.3 ml of minced tissue was mixed with sufficient Matrigel (Collaborative Research, Bedford, MA) to reach the total volume of 0.5 ml that was injected s.c. through a 16-gauge needle into 4-8-week-old male nude mice. Mice were obtained from the breeding colony of and housed in the Athymic Animal Facility of the Case Western Reserve University Cancer Research Center. Animals were housed 1 mouse/cage and fed autoclaved food ad libitum.

Mice were killed by ether anesthesia followed by cervical dislocation. The skins of the dead mice were carefully removed from the mice by blunt dissection. Any visible tumor was excised and bisected. One half of each tumor was fixed in formalin and embedded in paraffin. The other half was fixed in freshly prepared 2% paraformaldehyde at 4°C and embedded in glycol methacrylate with a modification of the method of Beckstead and Bainton (11) as described previously by us (12, 13).

As explained below, some minced tumor was injected into mice fresh and some minced tumor was cultured prior to injection as explants in 2.2 ml Eagles minimal essential medium with 20% calf serum (Grand Island Biological Company, Gaithersburg, MD) for 1-15 days in tissue culture flasks with a surface area of 25 cm² (Corning, Corning, NY). Cultures were maintained on a rocker platform (Belco Biotechnology, Vineland, NJ) that rocked them gently at a rate of 6 cycles/min in an environment of 5% carbon dioxide in 95% air. Medium on cultures was changed at intervals of 2-3 days. Fragments for injection into mice were dislodged from the surface of the culture flask by vigorously directing a stream of medium from a pipet against the fragments. These tissue fragments were removed from the culture flasks with wide-bore pipets, the excess medium was removed with suction, and the fragments were resuspended to a final volume of 0.5 ml in Matrigel at 4°C. All injections of cells or minced tumor were s.c. in the interscapular space.

Results

Animals that were given graded doses of PC-3 cells were observed for 3 months or until they were killed with tumors that were large and, in every case, verified histologically. There was a marked difference in the development of tumors (Fig. 1) between animals that received PC-3 cells in Matrigel and animals that received PC-3 cells in culture medium. All animals that received 20 or more PC-3 cells in Matrigel developed tumors. Only animals that received at least 0.5 million cells in culture medium without Matrigel developed tumors. During the 3-month period of observation, 25,000-fold fewer PC-3 cells were required to produce tumors when the vehicle for injection was Matrigel as compared with culture medium.

Experiments that are still in progress suggest that the data for LNCaP and DU 145 will be consistent with those described in the preceding paragraph. For histologically verified tumors, it appears that 7000-fold fewer DU 145 cells are required for the development of tumors when the period of observation is 10 weeks; animals that have not developed tumors are still under observation. Work with LNCaP has been more limited. We have never succeeded in producing tumors in nude mice by the injection of LNCaP in the absence of Matrigel. We did obtain tumor with the injection of 3 million cells in 0.5 ml of Matrigel; however, this observation is limited to one animal,
transplantation of carcinomas with histopathological patterns typical of human prostatic carcinomas (Figs. 2-4). They also expressed prostate-specific antigen (Fig. 4), acid phosphatase, and hexosaminidase (15) in patterns characteristic of human prostate cancer. All 6 successful xenografts expressed prostate-specific antigen; however, in some of these xenografts the expression of this antigen was heterogeneous; i.e., some portions of some tumors stained intensely while other portions of the same tumors stained lightly or not at all.

The 6 tumors that were transplanted successfully as verified histologically grew slowly. Tumors were not detectable in some cases for as long as 20 days after transplantation. The most slowly growing of these 6 tumors reached a volume of 500 mm$^3$ when the animal was killed 123 days after transplantation; the most rapidly growing tumor reached a volume of 790 mm$^3$ when the animal was killed 70 days after transplantation. The interpretation of these measurements should be made in light of the knowledge that microscopically these tumors contained both neoplastic proliferations and organized Matrigel. In some cases, the tumors had begun to invade outside of the Matrigel. In a small number of cases when either primary tumors or cell lines were injected with Matrigel, organized Matrigel could be

and animals that have received fewer LNCaP cells in Matrigel have not developed tumors. Nagle et al. (14) found LNCaP to be less tumorigenic in SCID mice than three other lines of human prostatic carcinoma cells studied by them. In limited numbers of experiments, LNCaP was successfully transplanted both with and without Matrigel when 0.3–0.5 ml of minced tumor was injected s.c. into 6–8-week-old male nude mice.

Ten minced primary human prostatic carcinomas were injected into male nude mice fresh or after culture as explants for 1–15 days. Mice were not given exogenous immunsuppressive or hormonal treatments. Mice were killed 70–181 days after the transplantation of tumors. Tumors were examined histologically. Injection of 6 of the 10 tumors resulted in the successful

Fig. 1. Tumors at 3 months after injection of PC-3 cells into nude mice in Matrigel or in culture medium (no Matrigel). Animals that did (○) and did not (□) develop tumor in each group are separated by horizontal lines. Animals developed tumors after the injection of as few as 20 cells in Matrigel: the single animal that received PC-3 cells in Matrigel without the development of a tumor received 4 PC-3 cells in Matrigel. When cells were injected in medium without Matrigel, a minimum of 500,000 PC-3 cells were required for the production of tumors.

Fig. 2. A xenograft from a nude mouse after transplantation of fragments of primary prostatic carcinoma in Matrigel. The morphology shows pseudoglandular structures and occasional cribiform patterns. H & E. × 575.

Fig. 3. A xenograft from a nude mouse after the transplantation of fragments of primary prostatic carcinoma in Matrigel. Large clear cells typical of less well differentiated prostatic carcinoma show frequent mitoses one of which is markedly abnormal (arrow). A, a relatively low magnification view to show prostatic carcinoma (right half) invading contiguous collagenous connective tissue (left half) with an infiltrate of host cells of lymphoid morphology close to the interface between connective tissue and growing tumor. B, higher magnification of a portion of the tumor to show nests of clear cells, the same conspicuously abnormal mitotic figure (arrow), and two other mitotic figures (arrowheads). H & E. A, original magnification, × 450; B, original magnification, × 720.
found in the absence of neoplastic cells 3–5 months after transplantation; however, in the majority of cases, when the transplantation of cell lines or primary tumor was unsuccessful after 3–5 months, no s.c. masses could be measured externally or found at the autopsy of the animal. Experiments are in progress to determine if these xenografts can be transplanted serially.

Discussion

We have shown that the injection of human prostatic carcinoma cells suspended in Matrigel results in the growth of tumors at defined intervals after injection with 25,000- to 7,000-fold fewer cells than are required to produce tumors when the same cell lines are injected in the absence of Matrigel.

When reviewing the transplantation of human prostatic tumors into nude mice, Gittes (8) noted that the “...incidence of ‘take’ of prostate cancer is close to zero. The few cases reported as accepted have been anaplastic...except for a single line established temporarily by Reid. But she noted that the single success resulted from implanting over 100 specimens.” Describing his own experience, Gittes (8) stated that his laboratory “had no takes of prostate cancer” in their attempts to transplant human prostatic carcinomas in “over 50 tumors” transplanted into “several hundred mice with or without testosterone pellets.” Hoehn et al. (5) state that they “...examined 80 fragments of human prostatic carcinoma tissue heterotransplanted in nude mice...[of which] one graft, PC EW, survives as a serially transplantable line.” Some of these lines have been useful for experimental therapeutics (16).

van Steenbrugge et al. (16) have summarized the characterization of five transplantable prostatic carcinomas that they have established by direct transplantation into nude mice. Two of these, PC-82 and PC-135, were the result of transplantation of primary prostatic carcinomas; three, PC-EW, PC-133, and the Honda tumor, were obtained by the transplantation of prostatic carcinomas obtained from metastases in humans. One of these, PC-82, will not grow to form long-term cultures and has retained many of its original characteristics after 7 years of being passaged in nude mice. These retained characteristics include: the histological pattern; “adenocarcinoma-cribriform pattern”; a slow growth rate [doubling time of 18 ± 5 (SD) days after many passages in nude mice]; hormone dependence; and the expression of both prostatic acid phosphatase and nuclear androgen receptors.

Like Gittes, we found that many months were usually required for the appearance of definite tumors. Because the first two animals that we examined had only a mass of organized Matrigel that contained an occasional nest of benign-appearing prostatic ducts some of which had undergone squamous metaplasia, i.e., they demonstrated no evidence of neoplasia, we killed the remaining animals that had received the first 10 transplantations of tumors in order to examine the palpable tumors microscopically. We were surprised to find that the tumors of approximately one-half of our animals consisted of viable nodules of human prostatic carcinoma in addition to the vascularized, organized Matrigel.

The studies that we describe in this paper were carried out in the absence of exogenous androgens. Preliminary data in our laboratory suggest that the use of s.c. implanted pellets that contain several doses of sustained-release testosterone affects the successful transplantation of these tumors into male nude mice only infrequently. Additional preliminary data have shown no advantage in using SCID mice as compared with nude mice for the transplantation of human prostatic carcinoma; however, these studies involve only a small number of prostate cancers and must be repeated before they can be considered conclusive.

There is a great need for the development of new approaches to the medical therapy of prostatic carcinoma. Fifty years after the discovery that androgen deprivation causes (17) “significant improvement” in some patients with prostatic carcinoma, there are excellent contemporary studies (18, 19) that show that certain kinds of hormonal manipulation with or without antineoplastic chemotherapeutic agents are more effective than others in the treatment of metastatic prostatic carcinoma; however, we are unaware of any well designed, prospective study that demonstrates that life is prolonged in any subpopulation of patients with prostatic carcinoma by hormonal manipulation as compared with treatment with a placebo. Similarly, in the not too far distant past, a critical review of the chemotherapy of prostatic carcinoma led Tannock (20) to conclude that “There is no evidence that chemotherapy causes a meaningful prolongation of survival [in prostatic carcinoma].” If there are unique features of the relative refractoriness to medical therapy of prostatic carcinoma cells that are related to either species or organ, techniques that will permit the long-term growth of a large proportion of human prostatic carcinomas may facilitate the testing of new agents and new therapeutic regimens for the treatment of prostatic carcinoma.

References

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