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Communication

Primary Neoplastic Transformation *in Vivo* of Xenogeneic Skin Grafts on Nude Mice[1]

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ABSTRACT

Rabbit skin was infected with Shope papilloma virus and grafted orthotopically to nude mice. Typical Shope rabbit papillomas developed in most of the grafts, but similarly treated nude mouse skin grafts were not altered. These results demonstrated that xenogeneic tissues retained susceptibility to oncogenic agents after transplantation to nude mice. The results also implied that it should be feasible to study carcinogenesis of human tissues in this system.

INTRODUCTION

Athymic nude mice are unable to reject grafts of xenogeneic tissues as a result of greatly impaired immunological responses (4). These animals are of major interest since they can provide an environment for the growth of transplants of normal and neoplastic cells which is devoid of the usual intra- and interspecies immunological restrictions. The principal uses of nude mice have included propagation of human tumors (2, 3) and assay of tumorigenicity of cells "transformed" *in vitro* after treatment with oncogenic agents (11). Nude mice have also provided information on the role of cellular immunity to oncogenesis (1, 12, 13). In this report, we demonstrate a different and unique application of nude mice, that of providing a neutral environment for the direct neoplastic transformation of xenogeneic tissues with oncogenic agents. The methods described are simple and convenient and may be immediately applicable to the assessment of carcinogenic chemicals and oncogenic viruses in various human tissues.

MATERIALS AND METHODS

Noninbred Swiss nude mice (nu/nu) of both sexes were purchased from Harlan Industries, Inc., Indianapolis, Ind. They were fed autoclaved laboratory chow and water and housed in filter bonnet cages in a room with restricted access. New Zealand White rabbits of both sexes were obtained from Dutchland, Inc., Denver, Pa. Conventional laboratory chow for the rabbits was supplemented with free access to mineral salt blocks and weekly fresh kale. SPV[4] was prepared by homogenization of cottontail rabbit papillomas (Earl Johnson, Rago, Kans.) at 10% (w/v) ratio in 0.68% NaCl-0.17% Na_2HPO_4-KH_2PO_4 (pH 7.4), and collection of the supernatant was obtained by low-speed centrifugation (6). Rabbit skin was obtained from the shaved surface of the dorsal surfaces of the pinnae. Split-thickness skin grafts (0.5 × 4.0 × 4.0 mm) were repeatedly punctured with a scalpel point, immersed in SPV suspension, and incubated for 1 hr at 37°.

Under pentobarbital anesthesia, graft beds were prepared on the left dorsolateral thorax of nude mice. The skin at these sites was elevated with a toothed forceps and snipped off with a curved scissors to prepare beds. The underlying highly vascular panniculus carnosus was carefully preserved. Grafts of rabbit or nude mouse skin (4 × 4 mm) were fitted to these beds, and excess fluid was removed by blotting with a sterile gauze pad. The grafts were secured with a Vaseline petroleum jelly-coated gauze pad affixed to an adhesive dressing (Sheer Spots; Johnson and Johnson, New Brunswick, N. J.). Occlusive dressings consisting of a gauze pad fragment were overlaid with adhesive tape which was wrapped securely around the thorax. Dressings were removed after 7 days, and the grafts subsequently were inspected weekly.

RESULTS

A total of 4 experiments was conducted (Table 1). Eleven of 12 rabbit skin grafts inoculated with SPV developed tumors typical of Shope papillomas (Fig. 1). The papillomas grew to a maximum dimension of about 1.0 × 1.0 cm and survived as long as the host mice. A few animals have survived at least 15 weeks after grafting. Most of the transplant recipients developed severe wasting and either died or were sacrificed moribund within a month of transplantation (3 to 4.5 months of age). Autopsy revealed extensive involvement of liver, brain, and lungs with lesions typical of mouse hepatitis virus infection (10). The liver showed varying degrees of hepatocellular necrosis, ranging from moderate to severe, and extensive phlebitis was noted in brain and lungs. The murine hepatitis was a local epidemic in our colony, unassociated with the SPV inoculations.

The histological appearance of the papillomas was typical of the Shope rabbit papilloma (Fig. 2). The tumors were composed of acanthotic, hyperkeratotic, papillary fronds projecting vertically from a common base. All epidermal layers were hyperplastic; mitotic figures were abundant.

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[4] The abbreviation used is: SPV, Shope papilloma virus.
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Table 1

Development of Shope papillomas in SPV-infected rabbit or nude mouse skin grafted to nude mice

In each experiment, a separate donor rabbit was used.

	Papillomas/grafts	
Experiment	Rabbit skin	Nude mouse skin
1	3/3	
2	3/4	0/3
3	5/5	0/5
Total	11/12	0/8

There were no significant leukocytic infiltrates. None of the nude mouse skin grafts developed papillomatous changes (Table 1).

The longest surviving mouse in the study developed a locally invasive epidermoid carcinoma. The carcinoma completely replaced the papilloma and infiltrated the adjacent skin and muscle (Figs. 3 and 4). No metastases were found in regional lymph nodes or viscera. Malignant transformation is common in Shope papillomas resident in rabbits for >1 year. Development of malignant cells after only 4 months is highly unusual and may suggest a role for tumor immunity in retarding tumor progression in the Shope papilloma-carcinoma complex (5).

DISCUSSION

In this report we demonstrate for the first time that the nude mouse will support the neoplastic transformation of xenogeneic tissue by an oncogenic agent. Specifically, normal rabbit skin was transformed to Shope papilloma after SPV infection. The observation that nude mouse skin was not altered by the virus clearly demonstrates that the papillomas which developed were of rabbit, not mouse, origin. Papillomatous transformation of rabbit skin confirms our previous findings in which rabbit skin was similarly altered after grafting to orthotopic (5) or heterotopic (6) hamster cheekpouches or after grafting to the dorsum of antithymocyte serum-treated conventional mice (8).

The broader and more significant inference from our study is that similar approaches might provide an important method of assessing the long-term effects of carcinogenic chemicals, oncogenic viruses, and physical agents on human skin or other human tissues. Normal human skin has persisted after grafting to nude mice (7, 9). Comparisons of the sensitivity of human tissues with rodent tissues placed on nude mice should permit direct evaluation of the relative susceptibility of human and rodent tissues to putative human oncogens. Present approaches rely heavily upon the assumption that assays in laboratory rodent models accurately identify potential risks to man. Transformation of human cells in culture with oncogens is an alternative to rodent testing. Emergence of transformed lines sometimes requires protracted culture and repeated passage, and there is a risk of contamination with extraneous agents such as viruses, mycoplasmas, or tumorigenic cell lines. Further, transformed cells emerging from such studies may not be tumorigenic (11). An additional complication is the difficulty of propagating *in vitro* pure populations of appropriate specialized epithelia such as mammary gland, liver, prostate, and urinary bladder. Such cells are initially available only in relatively small numbers and may not persist when fibroblasts are also present. Indeed, such approaches have been applied to attempts to transform rabbit epidermal cells with SPV; as yet, despite numerous attempts, no tumorigenic transformed cell lines have ever been reported.

The principal limitation to the use of nude mice as a component of a carcinogenesis assay system is their fragile state of health. The susceptibility of these animals to latent murine viruses, such as murine hepatitis and Sendai viruses (10), severely limits their life span, especially when they have been housed in inadequate facilities (4), and remains a significant limitation on their practical value in long-term experiments.

In summary, the simple and convenient neoplastic transformation of rabbit skin grafted to the nude mouse permits the logical inference that such an approach will be ultimately useful in determining the susceptibility of human tissue grafts to oncogenic agents.

REFERENCES

1. Allison, A. C., Monga, J. N., and Hammond, V. Increased Susceptibility to Virus Oncogenesis of Congenitally Thymus-deprived Nude Mice. Nature, *252:* 746–747, 1974.
2. Bastert, G., Schmidt-Matthiesen, H., Michel, R. T., Fortmeyer, H. P., Sturm, R., Nord, D., and Gerner, R. Human Mammary Cancer in nu/nu Mice. A Model for Testing in Research and Clinic. Klin. Wochschr., *55:* 83–84, 1977.
3. Fogh, J., Fogh, J., and Orfeo, T. One Hundred and Twenty-seven Cultured Human Tumor Cell Lines Producing Tumors in Nude Mice. J. Natl. Cancer Inst., *59:* 221–226, 1977.
4. Gullino, P. M., Ediger, R. D., Giovanella, B., Merchant, B., Outzen, H. C., Reed, N. D., and Wortis, H. H. Guide for the Care and Use of the Nude (Thymus-deficient) Mouse in Biomedical Research. ILAR News, *19:* M1–M20, 1976.
5. Kreider, J. W. Studies on the Mechanism Responsible for the Spontaneous Regression of the Shope Rabbit Papilloma. Cancer Res., *23:* 1593–1599, 1963.
6. Kreider, J. W., Haft, H. M., and Roode, P. B. Growth of Human Skin on the Hamster. J. Invest. Dermatol., *57:* 66–71, 1971.
7. Kruger, G. G., Manning, D. D., Malouf, J., and Ogden, B. Long-term Maintenance of Psoriatic Human Skin on Congenitally Athymic (Nude) Mice. J. Invest. Dermatol., *64:* 307–312, 1975.
8. Pass, F., Niimura, M., and Kreider, J. W. Prolonged Survival of Human Skin Xenografts on Antithymocyte Serum-treated Mice: Failure to Produce Verrucae by Inoculation with Extracts of Human Warts. J. Invest. Dermatol., *61:* 371–374, 1973.
9. Rygaard, J. Skin Grafts in Nude Mice. 3. Fate of Grafts from Man and Donors of Other Taxonomic Classes. Acta Pathol. Microbiol. Scand., *82:* 105–112, 1974.
10. Sharkey, F. E. Histopathologic Observations on a Nude Mouse Colony. *In:* J. Fogh and B. C. Giovanella (eds.), The Nude Mouse in Experimental and Clinical Research, pp. 75–93. New York: Academic Press, Inc., 1978.
11. Stiles, C. D., Desmond, W., Sato, G., and Saier, M. H. Failure of Human Cells Transformed by Simian Virus 40 to Form Tumors in Athymic Nude Mice. Proc. Natl. Acad. Sci. U. S., *72:* 4971–4975, 1975.
12. Stutman, O. Tumor Development after 3-Methylcholanthrene in Immunologically Deficient Athymic-nude Mice. Science, *183:* 534–536, 1974.
13. Stutman, O. Tumor Development after Polyoma Infection in Athymic Nude Mice. J. Immunol., *114:* 1213–1217, 1975.

Fig. 1. Shope rabbit papilloma which developed in SPV-infected rabbit skin. Photograph was taken 21 days after grafting.

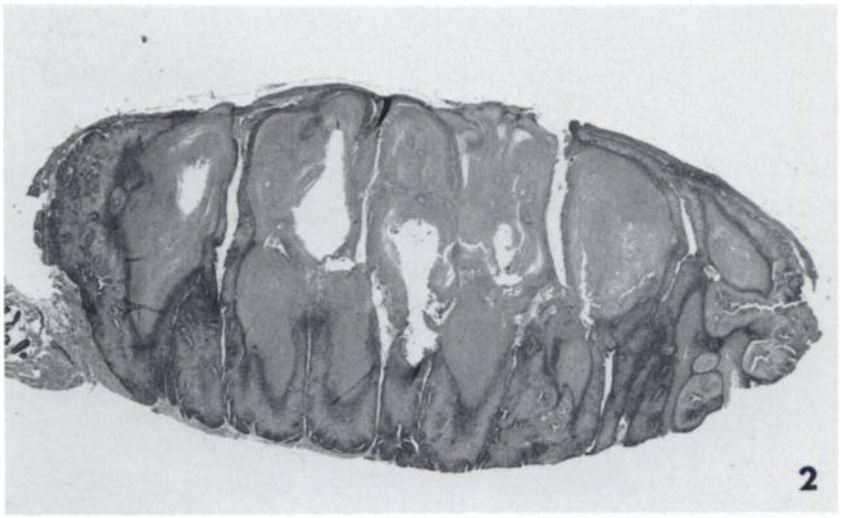
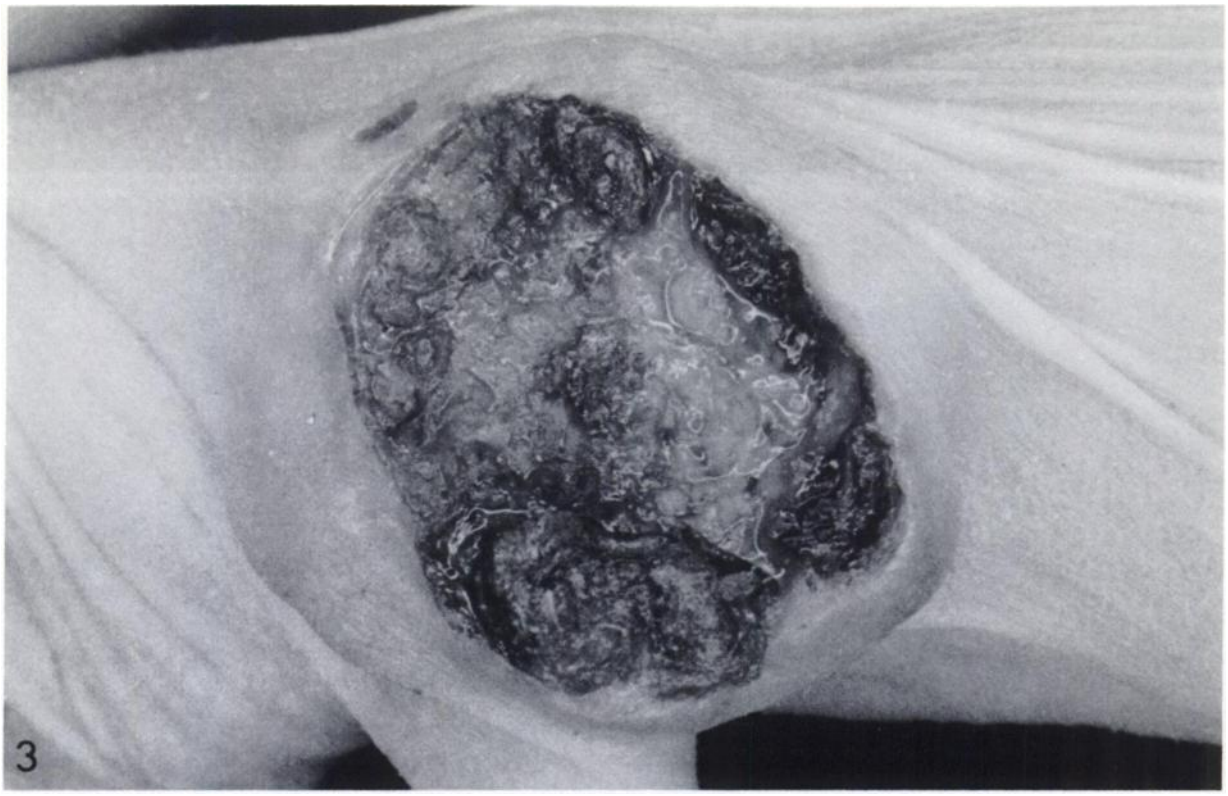


Fig. 2. Microscopic section of papilloma from Fig. 1. Papillary fronds of tumor interdigitate with markedly hyperkeratotic stratum corneum. H & E, × 12.75.



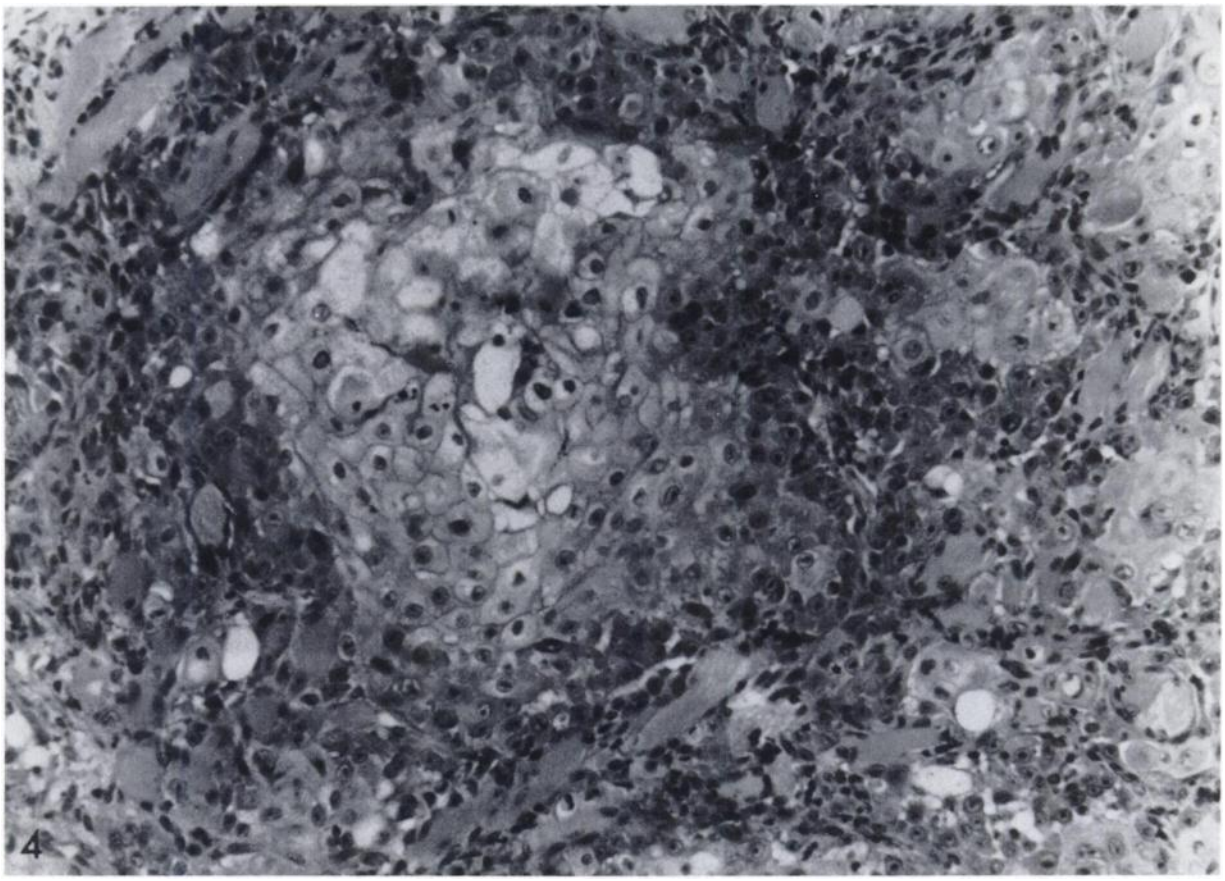


Fig. 3. Epidermoid carcinoma which developed in Shope rabbit papilloma. Photograph was taken approximately 4 months after grafting of SPV-infected rabbit skin. An ulceration with an indurated, rolled margin and a shaggy, exudate-covered base has completely replaced the papilloma. Superficial eschar is present at the periphery.

Fig. 4. Microscopic section of tumor from Fig. 3. Sheets of polygonal squamous cells infiltrate skeletal muscle bundles. A large focus of keratin pearl-like differentiation is in the center of the field.

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