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The Potential of the Nude Mouse Xenograft Model for the Study of Head and Neck Cancer*

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Summary. A total of 130 human head and neck cancers was transplanted subcutaneously in athymic nude mice in order to obtain a series of xenografts. All tumours were derived from previously untreated patients. Initial growth, which was histopathologically confirmed, was observed in 34 cases (26.1%). Serial passages were successful in 12 of 23 cases (52.1%). Of 117 squamous cell carcinomas, 30 (25.9%) showed initial take in the mice. Poorly differentiated squamous cell carcinomas tend to grow more readily than moderately differentiated and well-differentiated ones. Material from metastatic lymph nodes tends to show a higher take than material from primary tumours. In general the tumour-volume doubling time decreased to 4–6 days when the number of passages increased. Histology of the xenografted tumours showed that transplantation had caused no major changes. No macro-, or microscopic signs of metastasis were observed in any of the mice. The implications of this model for fundamental and applied research are discussed.

Key words: Neoplasma transplantation – Head and neck cancer – Xenografts – Athymic mice – Nude mice

Introduction

Athymic nude mice lack a thymus-dependent immune system and various tumours can be successfully transplanted and serially grown in these animals [4, 6, 23]. This xenograft model seems promising for the study of tumour cell biology and the evaluation of anti-cancer therapies. The human origin of the xenografted tumour cells is preserved according to the results of chromosome analysis [27], LDH isoenzyme analysis [19] and the expression of organ-specific neo-antigens [25].

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However the extent to which characteristics of the tumour providing the xenograft are maintained still has to be settled. The transplanted tumour grows faster when the number of passages is increased [10, 15]. Metastasis and invasion, characteristics of malignant tumour growth, are rarely found [21].

The success of initial and serial takes varies among tumour categories [4, 19]. Little is known about the take of human head and neck tumours in this model. This study describes a group of 130 head and neck tumours which have been transplanted in nude mice during the last 4 years. Take rate and growth pattern are analysed in primary and serial passages. The question as to whether it is possible to use this model to test anti-cancer therapies is discussed.

Materials and Methods

Animals

Female nude mice (B10.LP/Cpb, 8–10 weeks old) were obtained from the Centraal Proefdierenbedrijf TNO (Zeist, The Netherlands). The mice were kept under specific pathogen-free conditions. The cages were covered with paper filters and all manipulations were conducted under a laminar-flow cabinet. Cages, bedding, food (1210, Hope Farms, Woerden, The Netherlands) and water were autoclaved before use. The water was brought to a pH of 2.8 with 0.1 N HCl and contained no antibiotics. The temperature in the animal room ranged from 24° to 28° C and the humidity from 55% to 65%.

Tumours and Methods of Implantation

Only head and neck tumours from previously untreated patients were selected for implantation. Tumour specimens were removed aseptically and collected in ice-cold Hanks' buffered salt solution with 200 U/ml penicillin and 200 μg/ml streptomycin. Slices measuring 3 × 3 × 1 mm were dissected and implanted subcutaneously in the lateral thoracic region on both sides of the animal. Human tumours growing in nude mice and measuring between 800 and 1,000 mm^3 were serially transplanted in a similar way. Tumour material was cryopreserved in liquid nitrogen using McCoy's medium (Gibco, Hoofddorp, The Netherlands) with 20% fetal calf serum (Gibco) and 10% dimethyl sulphoxide.

Tumour Take and Measurement

Tumour take was defined as the presence of a histopathologically confirmed tumour of increasing size. When a tumour was capable of surviving more than four passages, a tumour line was established. Growth was measured bi-weekly using vernier calipers. Tumour volume was calculated as length × width × height × 0.5 [14]. Tumour growth curves from the animals were plotted individually on semilog graph paper.

Histopathology

When the animals had been killed, all internal organs were inspected macroscopically, special attention being given to the regional lymph nodes. Each organ showing an abnormal macroscopic appearance was excised and examined microscopically. In a proportion of mice axillary lymph nodes,

Table 1. Take of various types of head and neck tumours in nude mice

Tumour type	No. of tumour takes/ no. transplanted	No. of serially grown tumours/no. attempted
Squamous cell carcinoma	30/116 (25.9%)	11/22[a] (50.0%)
Salivary gland	2/9 (22.2%)	1/1
Chondrosarcoma	0/1	0/0
Non-Hodgkin lymphoma	0/1	0/0
Mucosal melanoma	2/2	0/0
Total	34/130 (26.1%)	12/23 (52.1%)

[a] Two lines were lost after the fifth passage

Table 2. Take rate of squamous cell carcinomas of the head and neck according to their degree of differentiation

Degree of differentiation	No. of tumour takes/ no. transplanted	No. of serially grown tumours/no. attempted
Poorly	7/13 (53.8%)	3/4 (75.0%)
Moderately	12/44 (27.3%)	3/7[a] (42.8%)
Well	11/59 (18.6%)	5/11[a] (45.4%)

[a] One line was lost after the fifth passage

kidneys, lungs, spleens and sections of small intestine and liver were investigated histologically in order to detect metastatic tumour foci and histopathological changes. Material for microscopic examination was fixed in 4% neutrally buffered formalin and processed for paraffin embedding. Sections of 5 μm were stained with haematoxylin and eosin. At the time of each subpassage representative tumour slices were taken for microscopy. Histopathologic comparison was made between the mouse-grown tumour and its corresponding original human tumour. Attention was also paid to the degree of differentiation, necrosis and invasion of adjacent mouse tissue. Grades of differentiation were classified according to a four-grade scale: well, moderately and poorly differentiated squamous cell carcinomas, and undifferentiated carcinomas. When a tumour had varying degrees of differentiation, it was decided that the less differentiated parts were representative for that tumour.

Results

A total of 130 head and neck tumours, derived from previously untreated patients, were transplanted into athymic nude mice. Tumour growth in the mouse was observed in 34 cases (26.1%) (Table 1). For squamous cell carcinomas, with 116 tumours the largest group, the initial take rate was 25.9%. Attempts have been made to establish tumour lines for these xenografts. So far, this has been succesful in 12 of 23 (52.1%) cases. Two tumour lines were lost after the fifth passage. Poorly differentiated squamous cell carcinomas tended to take better than moderately and well-differentiated ones (Table 2). Tumours derived from the hypopharynx grew more readily than those from other mucosal

Table 3. Take rate of squamous cell carcinomas according to their localization within the head and neck

Localization	No. of tumour takes/ no. transplanted	No. of serially grown tumours/no. attempted
Oral cavity	13/59 (22.0%)	7/11[a] (63.6%)
Larynx	6/24 (25.0%)	0/4
Oropharynx	4/16 (25.0%)	0/3
Hypopharynx	5/9 (55.5%)	3/3[a]
Nasopharynx	0/4	0/0
Skin	1/2	1/1
Unknown primary site	1/2	0/0
Total	30/116 (25.8%)	11/22 (50.0%)

[a] One line was lost after the fifth passage

Table 4. Take rate of head and neck tumours derived from primary tumours and lymph-node and distant metastases

Site	No. of tumour takes/ no. transplanted	No. of serially grown tumours/no. attempted
Primary	27/117 (23.0%)	10/19[a] (52.6%)
Lymph node metastasis	6/11 (54.5%)	2/4[a] (50.0%)
Distant metastasis	1/2	0/0
Total	34/130 (26.1%)	12/23 (52.1%)

[a] One line was lost after the fifth passage

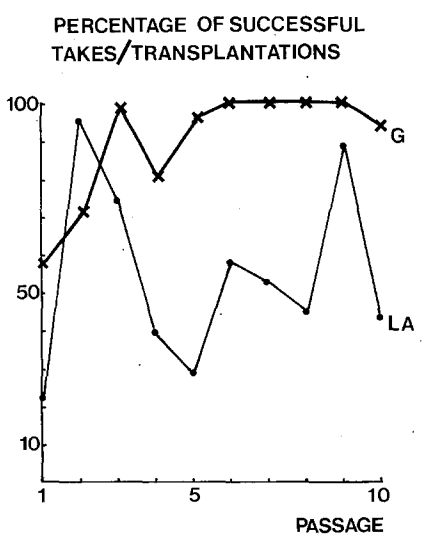

Fig. 1. Percentage of successful takes of attempted transplantations in subsequent passages for tumour line LA and G. At least 12 tumours were transplanted in each passage

Table 5. Characteristics of tumour lines

Line	Histology original tumours	Localisation of the original tumour in the patient[b]	Histology xenografts	Invasion in mouse
P	Well diff. squa. cell ca.	Alveolar process	Unchanged	−
LA	Poorly diff. squa. cell ca.	Piriform sinus	Small pseudosarcomatous areas	−
SG[a]	Mod. diff. squa. cell ca.	Piriform sinus	Unchanged	−
PV	Muco-epidermoid ca.	Mandibular gland	Unchanged	+
G	Well diff. squa. cell ca.	Skin of auricle	Small pseudosarcomatous areas	−
L[a]	Well diff. squa. cell ca.	Lymph node (floor of mouth)	Unchanged	−
H	Well diff. squa. cell ca.	Lymph node (tongue)	Unchanged	−
O	Mod. diff. squa. cell ca.	Palate	Small pseudosarcomatous areas	−
W	Mod. diff. squa. cell ca.	Floor of mouth	Small pseudosarcomatous areas	−
T-I	Well diff. squa. cell ca.	Cheek mucosa	Small pseudosarcomatous areas	−
KR	Poorly diff. squa. cell ca.	Floor of mouth	Unchanged	−
M-L	Poorly diff. squa. cell ca.	Post. wall hypopharynx	Unchanged	−

[a] These lines were lost after the fifth passage
[b] Localization of the primary tumour in parentheses
Mod. diff. squa. cell ca.: Moderately differented squamous cell carcinoma
Post.: Posterior

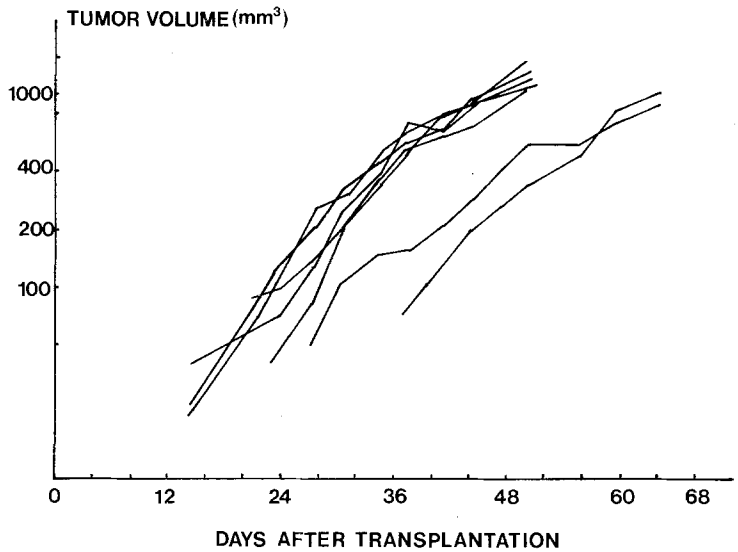

Fig. 2. Individual growth curves of seven xenografts in the second passage. The tumour was derived from patient KR

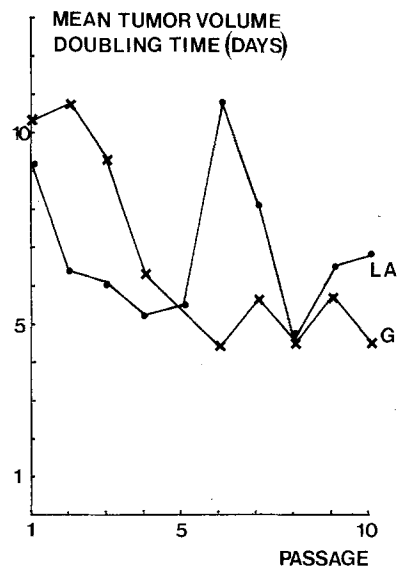

Fig. 3. Mean tumour-volume doubling time (100–200 mm^3) in subsequent passages for tumour lines LA and G. Data were obtained from at least seven tumours per passage

sites (Table 3). However, three of the nine hypopharyngeal tumours were poorly differentiated squamous cell carcinomas and all three tumours took initially. Tumour material from lymph-node metastasis tended to show initial growth more often than material from primary tumours (Table 4). The success rate in obtaining tumour lines from metastasized tumours was not increased. Takes were obtained from the same patient for metastasis of a melanoma and the

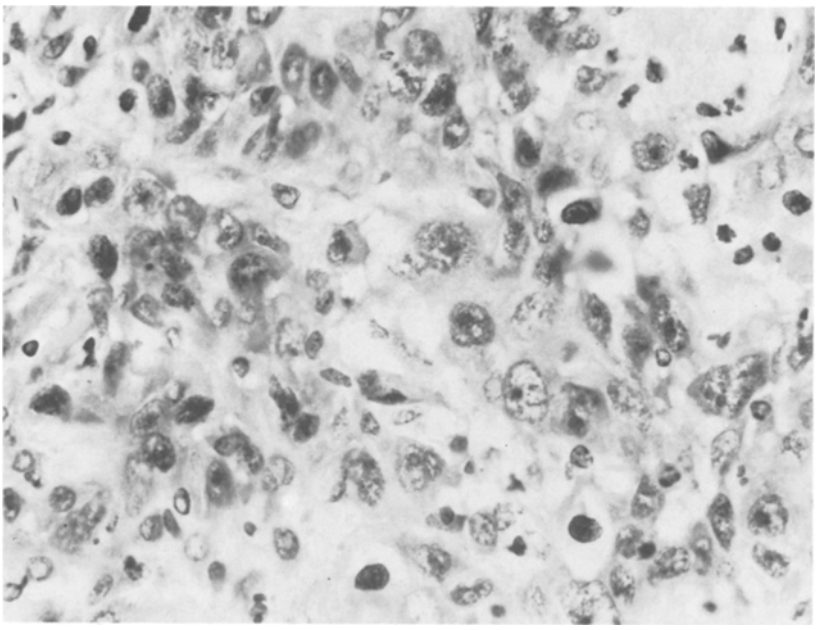

Fig. 4. Xenografted tumour of line LA (fifth passage) with pseudo-sarcomatous features. The epithelial character of the tissue is lost. (Original magnification × 400, HE)

primary tumour. The lymph-node metastasis of another patient grew successfully, whereas the primary tumour, which had been transplanted 3 months earlier, failed to grow.

In four [P, LA, PV and T-I (Table 5)] of six evaluable tumour lines the take rate in the subsequent passages varied between 30% and 100%. In two lines (G and W) a relatively constant take rate of 80%–100% was reached after two passages. Two examples of these characteristics are given in Fig. 1. The other six tumour lines could not be evaluated because the number of passages was too small. The mouse-grown tumour reached a volume of 100 mm^3 in 3–8 weeks and generally showed progressive growth, initally in an exponential manner; however, growth rates subsequently decreased (Fig. 2). In four tumour lines the tumour-volume doubling time from 100 to 200 mm^3 decreased after two to four passages to a constant level of 4–6 days. In two lines the tumour-volume doubling time varied between the individual passages from 5 to 11 days (Fig. 3).

Histological characteristics of the 12 tumour lines are summarized in Table 5. No major changes were observed after passaging in the nude mice. In five lines minor areas with pseudosarcomatous features were observed (Fig. 4). The nuclei were spindle-shaped and the epithelial character was lost.

Most tumours were well circumscribed and surrounded by a connective tissue capsule, originating from the mouse. Invasion in the adjacent mouse skin was observed in one line. Such invasion was found in half of the cases, but there was no invasion of muscles. No macroscopic or microscopic signs of metastasis to

regional lymph nodes, lung, spleen, kidney or liver could be found in ± 300 tumour-bearing animals.

Mouse-grown tumours of all lines showed necrosis. The extent varied between tumour lines and increased according to tumour volume. In one tumour line (W) only a thin rim of viable tumour tissue was left when the tumour reached a volume of 1,000 mm^3. The remaining non-viable tissue consisted of keratin fibres and areas with scattered pyknotic cells.

Discussion

From this study it has become clear that head and neck cancer can be grown in athymic nude mice. In 26% percent of the attempted transplantations tumour material started to grow subcutaneously in the mouse and serial passaging was possible in half of the cases. The intial take of 26% is lower than a mean value of 36%, obtained with various tumour types[1]. This can be explained by the fact that with head and neck tumours a relatively high risk of contamination from bacteria and viruses is present. Contamination in a transplanted tumour might result in a stimulation of the remaining immune system of the mouse, leading to an inhibition of tumour growth. Kyriazis et al. [13] showed that an infection with mouse hepatitis virus prevented tumour growth in the majority of nude mice.

That the remaining immune system can indeed play a role in inhibiting growth has been shown by Kopper et al. [12] who established an increase in tumour take rate by damaging the macrophages with carrageenan. Habu et al. [8] found an increase in tumour take by blocking natural killer cells with an anti-serum. Immune suppression, therefore, appears an attractive way to increase the take rate. Such an increase is required to make reduction of the number of transplantations feasible.

Poorly differentiated head and neck squamous cell carcinomas tend to take better than moderately and well-differentiated ones. This may be the result of a relatively higher proportion of stem cells in poorly differentiated head and neck tumours, since a tendency towards higher cloning efficiency has been shown for poorly differentiated squamous cell carcinomas using the soft agar technique of Hamburger and Salmon [17]. A simpler explanation for the better take rate in poorly differentiated carcinomas might be that these carcinomas contain more viable tumour cells than the more differentiated tumours, where parts of the tissue consist of non-dividing cells and keratin. Bastert et al. [2] found a higher take rate with connective tissue-poor breast carcinomas than with connective tissue-rich carcinomas.

The relative high take in carcinomas of the hypopharynx can be explained by the fact that three of these tumours were poorly differentiated. Material from lymph-node metastasis appears to take more readily than material from primary tumours. The higher take rate for material from metastatic tumours was also

1 List of human tumours transplanted to nude mice (1977) In: Nomura, T. et al. (eds) Proc 2nd Workshop on Nude Mice. University of Tokyo Press, Tokyo, pp 587–595

found by Fogh et al. [4] and Giovanella et al. [5] for various types of tumour. This correlation was not found when breast tumours were used [1, 2]. An explanation for the higher tendency of metastatic cells to grow in nude mice might be that the proliferation rate is higher in these cells than in these of the primary tumour. Absence of contamination in the metastatic material might also be important.

In four of six tumour lines the take rate varied in subsequent passages. A similar finding is reported by Houghton and Taylor [10], who established six colorectal tumour lines in immune-suppressed mice. Mattern et al. [16] found a higher percentage of take with increasing passages for breast, lung and ovarium tumours, as observed for two tumour lines in this study.

In four of six lines an increase in growth rate, measured as the decrease of the tumour-volume doubling time from 100 mm^3 to 200 mm^3, was seen during the first passages. Thereafter the tumour-volume doubling time varied between 4 and 6 days. This decrease in tumour-volume doubling time was also found for breast carcinomas [1], transplanted in immune-suppressed mice. Selection for faster growing cells or increase in the growth fraction might take place. An increase of cells in the S-phase was also found in the first passages when they were measured with flow cytofluorometry [15] and ^{3}H-thymidine uptake [25]. It must be added that in this material two of six tumour lines showed no increase in growth rate in subsequent passages. Fodstad et al. [3] observed no decrease in tumour-volume doubling times of melanomas with increasing numbers of passages.

The histology of the xenografts was generally similar to that of the original tumour. Giovanella et al. [5] found an increase in differentiation in 25% of their material from various parts of the body. Dedifferentiation is also reported for lung carcinomas [15] and breast carcinomas [21].

No signs of metastasis could be found in ± 300 tumour-bearing animals. In one tumour line invasion of the skin was observed in half of the transplanted tumours. The incidence of metastasis in the nude mice is low when using various types of tumour [22, 23]. The immune system of the nude mice can play an inhibiting role in the development of metastasis. In 3-week-old mice, which have low levels of natural killer cells, the incidence of metastasis is elevated [9].

It is possible with this model to obtain sufficient human head and neck tumour material outside the human body to enable fundamental and applied research on head and neck cancer. For other tumour types this model has already been shown to be of value in the field of tumour immunology. Thomson et al. [25] demonstrated the presence of organ-specific neo-antigens in various tumour types. This model can also contribute to the production of tumour-specific antisera [7].

Most studies with this model have been concerned with applied research into the effect of various treatment modalities. Attention has been focused particularly on chemotherapy. Xenografts generally respond to agents that are clinically effective [11, 18, 21]. The study of bronchial carcinomas [24] has demonstrated that the xenografts reproduced the chemotherapeutic response pattern of their source tumours. Therefore, this model appears to be of value as a screening model for new chemotherapeutic agents. Such a screening model is

also urgently needed for head and neck cancer. Further studies using this model are to be encouraged.

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