# THE PRODUCTION OF CLONES (TUMORS FROM ONE CELL) OF JENSEN'S SARCOMA

L. F. Sharlikova and U Min

Laboratory of Experimental Chemotherapy (Head – Corr. Member AMN SSSR Prof. L. F. Larionov, Dir. of Research – Candidate Med. Sci. G. L. Zhdanov) and Laboratory of Tissue Culture (Head – Active Member AMN SSSR A. D. Timofeevskii) of the Institute of Experimental and Clinical Oncology (Dir. – Active Member AMN SSSR N. N. Blokhin) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR A. D. Timofeevskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 3, pp. 85-88, March, 1961
Original article submitted April 11, 1960

The resistance of tumors to drugs is one of the current problems of the chemotherapy of malignant disease. Tumor variants resistant to the action of certain chemotherapeutic compounds have already been produced experimentally [4, 6]. We have obtained variants of sarcoma 45 of rats resistant to the chloroethylamines and ethylenimines [2, 3], and also variants of Jensen's sarcoma resistant to embichin. The latter tumor was found to be the most suitable model for the study of certain aspects of drug resistance, for it is highly sensitive not only to chloroethylamines and ethylenimines, but also, as our investigations have shown, to compounds of other classes and groups.

In order to approach the problem of the possible mechanism of development of drug resistance in tumors, we considered it to be of the utmost interest and importance to obtain clones derived from a single cell.

Attempts to obtain tumor clones have been made in the past by many workers [5, 7, 8], but these experiments were carried out mainly on ascites forms of transplantable tumors in animals and in experiments in vitro. For our investigations it was essential to obtain clones of a solid transplantable tumor – Jensen's sarcoma. This tumor was obtained in 1907 from a grey rat which was injected with the pseudobacillus of tuberculosis. This tumor, which is a polymorphocellular sarcoma, is practically 100% transmissible [9].

## EXPERIMENTAL METHOD

The material for inoculation of animals with a single tumor cell was prepared as follows. The original Jensen's sarcoma, freed from necrotic areas, was carefully minced with scissors in sterile conditions to obtain a homogeneous pulp. A 0.25% solution of trypsin was then added. The suspension was incubated at 37° for 30 minutes, and then centrifuged for 10 minutes at 1000 rpm. The supernatant fluid was removed and the residue was mixed with synthetic nutrient medium 199 to which calf serum had been added. The suspension of tumor cells in nutrient medium was diluted serially so that one drop of medium in the last dilutions contained single, isolated cells. With a fine capillary tube, under a binocular microscope, one cell was removed, clearly visible in the lumen of the capillary tube.

The skin of young rats was pierced with the same capillary tube, the contents of which were injected subcutaneously by blowing through a rubber tube as shown in Fig. 1, so that a small air bubble was formed beneath the skin together with the injection of the tumor cell. Most rats were inoculated subcutaneously in the dorsal region, and some in two places – in the right and left flanks.

Results of Inoculation of Young Rats with One Jensen Sarcoma Cell

| Experiment No. | Site of inoculation | Age of rats | Number of rats | Results of inoculation | Clone No. |
|---|---|---|---|---|---|
| 1 | Beneath the skin of the dorsal region | First hours of life | 16 | Tumor successfully inoculated in one rat | Clone No. I |
| 2 | The same | First hours of life to 4 days | 35 | Tumor not successfully inoculated | – |
| | " " | First hours of life | 20 | The same | – |
| 3 | " " | 1 day | 12 | " " | – |
| | " " | 2 days | 9 | " " | – |
| 4 | Beneath the skin of the right and left flanks | First hours of life | 20 | Tumor inoculated successfully in two rats | Clone No. II |
| | The same | 2 days | 9 | Tumor not successfully inoculated | Clone No. III |
| | Beneath the skin of the dorsal region | First hours of life | 9 | The same | – |
| | The same | 1 day | 7 | " " | – |
| | " " | 2 days | 14 | " " | – |
| | " " | 6 days | 14 | " " | – |

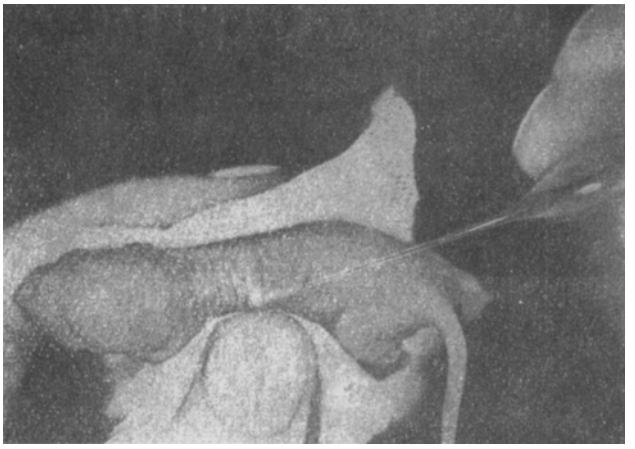

Fig. 1. Inoculation of new born rat with a single Jensen sarcoma cell.

The ages of the youngs rats selected for the experiment ranged from a few hours to six days.

## EXPERIMENTAL RESULTS

The results of the experiments are presented in the table.

It is clear from the table that altogether 165 rats were inoculated, 136 beneath the skin of the dorsal region and 29 in both flanks. Tumors developed in only three rats: in one animal inoculated beneath the skin of the dorsum (Fig. 2) and in two rats inoculated in both flanks, which each developed one tumor.

It must be pointed out that in all three successful cases the tumors developed in young rats inoculated during the first hours of life.

The developing tumors grew rapidly, and on the 25th-30th day after inoculation they had attained weights of 6.7, 1.5 and 15.3 g respectively, when they were transplanted into adult rats by the ordinary method.

Fig. 2. Young rat with tumor developing at the site of inoculation of one cell.

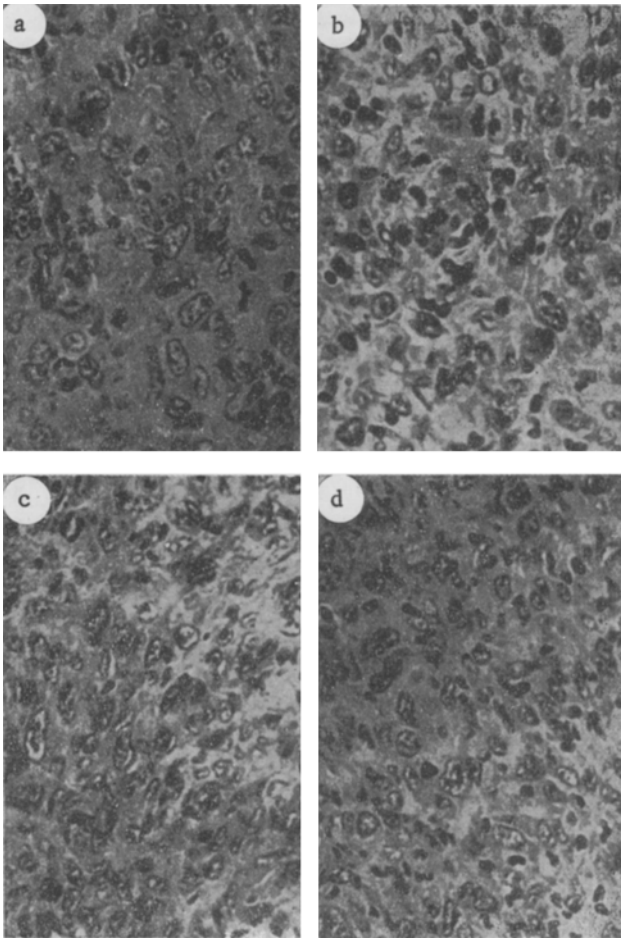


Fig. 3. Morphology of the original Jensen's sarcoma and of its three clones. a) Original Jensen sarcoma; b) clone I; c) clone II; d) clone III. Stained with hematoxylin-eosin. Magnification 400×.

Three clones I, II and III were thus obtained. The rate of successful inoculation of the tumors of all three clones into adult rats was subsequently 100%. The tumors grew very quickly and on the fourth day after transplantation their average diameter was 7-8 mm, and on the 18th-20th day their average weights were 48.6 g

(clone I), 50.4 g (clone II) and 42.3 g (clone III). Meanwhile the average weight of the original tumor at this period had reached 25-30 g. It should also be pointed out that the dimensions of the tumors of the different clones in the individual rats were very similar, especially in the first generations (low variability) which is not usually observed during transplantation of the original tumor.

Morphological examination of sections stained with hematoxylin-eosin showed no significant differences between the morphology of the three clones, as is observed when clones are obtained in tissue culture [1]. A photomicrograph of the original Jensen sarcoma and of its three clones is shown in Fig. 3.

Further investigations showed that the clones thus obtained differed from each other in their sensitivity to certain chemotherapeutic preparations such as, for example, embichin, omain and myleran.

## SUMMARY

In inoculating a single cell of Jensen's sarcoma to newborn ratlings three clones of this tumor were obtained. The clones grown by the usual method on adult animals were characterized by a more rapid growth than the initial Jensen's sarcoma. There were no significant morphological differences between the initial tumor and its clones.

## LITERATURE CITED

1. U Min, Voprosy Onkol. 7, 5 (1959).
2. L. F. Sharlikova, Voprosy Onkol. 6, 74 (1955).
3. L. F. Sharlikova, The Development of Drug Resistance in Sarcoma-45 of Rats to Chloroethylamines and Ethylenimines (Candidate's dissertation) [in Russian] (Moscow, 1958).
4. D. A. Clarke et al., Ann. (N. Y. Acad. Sci., 1954) 60, 2, 235.
5. K. Hosokawa, Gann. 41, 236 (1950); 42, 343 (1951).
6. H. Jackson, Brit. J. Cancer 8, 336 (1954).
7. G. Klein, Cancer Res. 19, 343 (1959).
8. S. Makino and J Kano, Nat. Cancer Inst. 15, 1165 (1955).
9. H. L. Stewart and S. M. Schlyen, Animals (Washington, 1959) p. 348.

---

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

---