Establishment and behavioral study of a tumor model in Balb/c mice for experimental cancer studies

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Abstract

The establishment of a standard and well-studied animal tumor model in order to evaluate the effects of new anti-cancer therapeutics and the study of tumor biology is crucial to cancer research institutes in Iran. In this study, we established a standard tumor model in Balb/c mice. After the evaluation of the available cell lines, WEHI-164 cells were used for tumor induction in male and female four-week inbred Balb/c mice.

On the day 0, mice were inoculated with $7 \times 10^6$ WEHI-164 cells subcutaneously and tumor dimensions were measured daily. Statistical analysis of the results indicated that there is no significant difference between male and female groups and the yield of 82% about tumor induction was determined. From the day 8, tumor regression was observed.

Keywords: in vivo, tumor model, WEHI-164, Balb/c mice.

Introduction

Many researchers have been trying to find out new methods for cancer treatment. In this way, a project has been started in Mashhad University of Medical Sciences applying a combination of two physical techniques: electroporation and photodynamic therapy. Electroporation is a new method with a ten-year history in which, electrical field is used for drug delivery to the cells (9). Photodynamic therapy has been performed for more than one hundred years. In photodynamic therapy, a light sensitive dye is introduced to the body and then, the tumor site is irradiated by appropriate light source. Activated dye can destroy the tumor cells (20). Side effects due to the distribution of dye in healthy tissues is one of the problems of this technique that limits its widespread application.

In this project, in order to decrease the dosage of dye, electrical pulses are to be used for increasing dye delivery into the tumor cells. Absence of an in vivo animal tumor model was found to be the first and most important problem. Unfortunately, no standard in vivo animal tumor model has been established in our country and there is no standard and well-established animal tumor model for preclinical studies. Therefore at the first step, a tumor model was established in Balb/c mice.

In this study, we established a standard animal tumor model using the available cell lines and laboratory animals. There are other methods to obtain tumor models too. These are chemical carcinogenesis (10, 11, 19), UV (14, 12), X (8, 4) and Gamma (15, 18) irradiation and subcutaneous implantation of tumor tissues (1).

These procedures have their own limitations. For example, chemical carcinogenesis and irradiation have low tumor yield and produce heterogeneous tumors. Implantation of tumor tissues is a laborious work and their preservation is difficult.
In this study, in order to obtain a standard tumor model, we used a special tumor cell line. In this method, at first, the cells were propagated and then, injected to animals. These cells proliferate in the mice body to develop tumor. The site and kind of injection are important. Subcutaneous injection of tumor cells causes subcutaneous solid tumors while; intravenous injection may cause cancer in visceral organs such as the lung (1).

Materials and methods

After the assessment of available cell lines in Iran, we concluded that the best cell line for tumor induction in Balb/c mice is WEHI-164. This cell line was obtained from the Pasteur Institute (Tehran, I.R. Iran) and then cultured. WEHI-164 (Iranian Cell Collection No: NCBI C200, ATCC No: CRL-1751, ECACC No: 94052601) is a continuous and adhesive cell line with an epithelial like morphology.

This cell line was originally established from a fibrosarcoma induced by subcutaneous injection of 3-methylcholanthrene in Balb/c mice (Figure 1).

WEHI-164 cells was cultured in RPMI-1640 medium containing 2 mM glutamine, 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin in presence of 5% CO₂ in 37°C. When the cells formed a monolayer at the bottom of culture flasks (~0.5 – 2) × 10⁸ cells/cm², they were detached by 0.25% trypsin/EDTA solution and transferred into new flasks. Culture medium was renewed every 2-3 days.

In the next step, 4-week-old male and female Balb/c mice were provided from Bou-Ali Research Institute - Mashhad. They were fed standard chow ad libitum and the day before the test, their right flanks were carefully shaved. On the day 0, mice were divided into two equal male and female groups. The cells were trypsinized, washed 3 times and their concentration and viability was measured using Neubauer haemacytometer and Trypan Blue dye. Mice were inoculated with 100 µl of cell suspension containing 7 × 10⁶ live cells subcutaneously on their right flanks. 100 µl of Hanks solution was injected into the left flank as control.

After developing the tumor (Figure 2), pathologic sections were obtained to confirm fibrosarcoma tumor. For daily evaluation of tumor volume, small diameter (a), large diameter (b) and tumor thickness (c) were measured by a Vernier’s caliper (with 0.02 mm accuracy). Tumor volume (V) was calculated according to the following formula (6): (Figures 3,4,5)

\[ V = \pi/6 (a.b.c) \]

Every pilot experiment was repeated several times in order to obtain exact calculation of statistical variables (mean volume of tumors and their standard deviations) and sample volume for in vivo experiments. SPSS software was used for statistical analysis. To determine statistically significant differences between the test groups (p < 0.05), we used the Duncan test.

Results

Results (Figures 6, 7, 8) obtained from this study show that tumor yield is about 82% and is the same in both male and female mice; i.e., from each ten mice, eight will develop appropriate tumor. No significant difference was found between male and female groups in tumor volumes. Statistical analysis show that the experiments require seven tumor mice in each test group (sample volume = 7, with 95% coefficient confidence). Tumor volume progressively increased until days 8-9 and then, decreased slowly. It seems that Tumor Necrosis Factor (TNF) secretion is responsible for this regression. (3)
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Figure 1: WEHI-164 cell line.

Figure 2: Shaved Balb/c mouse that developed tumor.

Figure 3: Measurement of large diameter of tumor.

Figure 4: Measurement of small diameter of tumor.

Figure 5: Measurement of tumor thickness.

Figure 6: Tumor volume changes in all mice.

Figure 7: Tumor volume changes in female mice.

Figure 8: Tumor volume changes in male mice.
Discussion

Although progression in cancer researches is promising in our country, most of the studies are still based on in vitro experiments. Since, the ultimate aim of all in vitro studies in clinical trials, an in vivo tumor model will bridge between in vitro results and clinical trials. Therefore, to obtain more reliable and reasonable results, this model should be investigated thoroughly and standardized. In the present study, we have established a standard and carefully investigated model that can be employed easily in in vivo cancer researches.

This tumor model can be employed in short-term studies on new anti-cancer treatments and in general, all the researches that tumor effects are appeared during 9 days, can benefit from this model (7). For extended studies in prolonged periods, other cell lines such as MOPC-315 (derived from a murine multiple myeloma) (2, 13, 17) and CT-26 (derived from a murine colon adenocarcinoma) (3) can be used with the same procedure to develop tumor in Balb/c mice. Finally, human tumor cell lines can be injected to nude mice to develop human tumors in mice (16, 5).

Acknowledgement

The authors thank Dr. Ali-Reza Khoee, assistant professor of the department of Pathology at Mashhad University of Medical Sciences who analyzed pathologic specimens and Mr. Reza Feyzi, M.Sc in Immunology at Bu-Ali Research Institute for his help and Mr. Saeed Ebrahim-Zadeh for his statistical analysis during the work.

References

Fibrosarcoma tumoral model