مقاله تحقیقی

ایجاد مدل‌های زنگ‌گرافت بومی و مشق شده از رده سلولی استاندارد گلیوبلاستوما مالتی‌فورمه در ایران

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چکیده

اء: گلیوبلاستوما مالتی‌فورمه یکی از شایع‌ترین و بدخیم‌ترین تومور آنتروپیسی بوده و نسبت به درمان‌های رایج مقاومت نشان می‌دهد. بهبود درمان بیماران مبتلایی به این تومور از میان بیماران مبتلا به پروگررسیون می‌باشد. در این مطالعه کاربرد روش های توتال و روش کنسرسیو در حال انجام و در ایران تزریق مطالعات در in vitro و در vivo مطالعه نشده است.

تومور در این مقاله است بر کلیه باید به توجه به شکل هورموتیپیک به موش‌ها تریبون کرد. سپس تومور مستقیم از همان اثر در این جراحی در زیر جلد موش‌ها کاشته شد. در این مطالعه موش‌ها از تهیه گردید و بر حسبGFAP و H&E پروآنتی‌ژنیکGFAP و H&E کد گیری کرده و سپس در in vivo مطالعات درمان‌های جدید این سرطان را مورد مطالعه قرار گرفتند.

کلمات کلیدی: گلیوبلاستوما، زنگ‌گرافت، موش‌های بومی، تزریق تیمومس
Establishment of Autochthonous & Standard Cell Line Derived Xenograft Models of Glioblastoma Multiform In Iran

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ABSTRACT

Background: GBM is the most common and malignant astrocytoma tumor and it is persistent to common treatment so, these patients have a very low survival. Several researchers around the world, including Iran, have been investigated GBM-cell line in vitro. However in vivo studies have not been fulfilled.

Methods: As standard cell line (U-87MG) derived from human GBM and total GBM tumor derived from 3 patients were heterotypically injected into 4-6 weeks old athymic nude mice. Pathologic investigation by H&E, GFAP and Ki-67 were examined 2 months post implantation.

Results: GBM characteristics appeared in H&E and GFAP and the rate of proliferation was 6% and in direct xenograft tumor was 9% which was consistent with the pathologic result of patient.

Conclusion: GBM Xengraft is the most suitable model for in vivo investigation and researcher can evaluate new treatments for this tumor. On the other hands, Pharmacogenomics differences in treatment response could be indicated among Iranians.

Keywords: Glioblastom, Xengraft, Tumor
INTRODUCTION

Glioblastoma multiform (GBM) is the most prevalent and malignant primary brain tumor and its incidence increases significantly among adults above 50 [1]. Virchow described this tumor for the first time in 1963 and then, Bailey and Cushing named it “Glioblastoma and spongioblastoma”. According to World Health Organization (WHO), astrocytic tumors are classified into four categories including GBM and grade IV [2]. Due to indeterminate boundary between tumoral and healthy brain tissues, the complete removal of the glioma high grade tumor is impossible. On the other hand, the sensitivity of these tumoral cells to chemotherapeutic drugs is low. There are also many limitations regarding the application of radiotherapy, as cerebral cells have a low endurance threshold for radiation. Therefore, the prognosis of this disease, despite significant developments in prognostic tools and using modern treatment equipments, is poor and the survival time of these patients is about one year [3].

As GBM has a high incidence in the world and does not respond properly to the treatment, several basic studies, both in vitro and in vivo, have been carried out in internationally recognized cancer research centers. In in vitro studies, cell lines derived from human GBM are cultured and proliferated in laboratory and the resultant cells are used in pharmacological research in order to investigate the effects of anti-cancer drugs. These cell lines are also marked with some drugs and then irradiated by various ray doses. Thereafter, an animal model is developed and the treatment factors used in in vitro phase of the study are evaluated in vivo. The xenograft models are accepted for the present time as the best animal models of cancer types. Nude mice without immune cells are used in basic cancer investigations and preclinical studies of new anti-cancer drugs.

Regarding the possibility of raising nude mice inside the country, it seemed necessary that first a GBM model of standard cell lines is developed and then the autochthonous cell lines of GBM are derived. The objective of this study was to develop a Glioma High grade xenograft model using simultaneously both standard cell lines and fresh tumoral tissue graft from native patients in order to facilitate basic research on central nervous system cancers with the help of these models. In addition the study aimed to study the sensitivity of both models versus routine chemotherapeutic drugs. The latter would be helpful in determination of pharmacogenomic differences in Iranian races.

METHODS

Animals
Male athymic nude mice of the age 4-6 weeks were used in this study. The mice were kept in microisolator cages in experimental tumor implantation laboratory of Imam Hospital, which is under supervision of Iran University of Medical Sciences (IUMS). These animals were fed ad libitum with autoclaved food and water. All phases of this investigation were planned and performed according to the internationally accepted ethical principles of working with laboratory animals.

Cell line
U-87MG, which is one of the cell lines of Glioblastoma multiform (GBM) was provided by the National Cell bank of Iran (NCBI) affiliated to Pasteur Institute. It was then cultured in RPMI 1640 culture medium containing 10% FBS.

Development of tumoral xenograft model from U-87MG cell line
To develop a xenograft model, $5 \times 10^6$ cells from the cell line of U-87MG in 200µl of RPMI 1640 culture medium without FBS were heterotopically and subcutaneously injected into the right flank region of nude mouse.

Development of tumoral xenograft model from primary human tumor
The primary GBM tumor specimens were obtained from three patients in the Shariati
Hospital of Tehran University of Medical Sciences, after they had signed the consent form. It is noteworthy that the nature of tumor was confirmed through MRI before and after surgery. The tumor specimens in RPMI 1640 sterile culture medium were transported to the experimental tumor implantation laboratory while they were stored in ice box, and there they were cut into pieces of the dimension 1×1×0.5 mm. For each patient’s tumor, five nude mice were anesthetized, according to the anesthesia protocol, by injecting the following dosages of drugs: 100 mg/kg of Ketamine 10% (Alfasan; Netherlands) and 10 mg/kg of Xylaznix 2% (Alfasan; Netherlands). Thereafter, a 5mm long incision was made on the skin of right and left flank region and a cut piece of the tumoral specimen was implanted subcutaneously in above-mentioned regions. Finally, the incision site was sutured with nylon 0-4 suture thread.

Figure 1 Tumor of GBM xenograft model

Pathological studies
Two months later, euthanasia was conducted on the mice in a human manner. The resultant tumors were removed from the animal skin, fixed in 10% buffered formalin and transported for further processing to pathology laboratory, where some slides were made of the specimens by H&E staining and also immunohistochemical staining with GFAP and Ki-67 (made of the Danish company DAkO). A pathologist then studied the slides.

RESULTS

Histopathologic studies by applying H&E staining on U-87MG cell line and on the tumoral graft to be transferred directly to nude mouse indicated the increased cellularity with many mitochondria. The nuclei showed also strong polymorphism. Vascular proliferation and numerous cases of necrosis were the other histopathologic characteristics observed. Necrotic zones were surrounded by anaplastic cells. In pathology, this histopathological scene is denominated “pseudopalisading necrosis” and is the hallmark of Glioblastoma multiforme. The immunohistochemistry (IHC) results gave evidence to positive Glial fibrillary acidic protein (GFAP), which is a proper marker for astrocytic tumors. The proliferation level of tumoral cells was assessed through Ki-67 marker. This marker indicated the increase of tumoral cells in the cell line by 6% and in the direct graft of human tumoral tissue by 9%, which correlated with the proliferation level of patients’ real tumors.
GBM is the most prevalent astrocytic tumor and despite using standard surgical, chemotherapeutic and radiotherapeutic treatment methods, the survival time after beginning of treatment is between one and at most two years [4]. Therefore, developing animal models of this disease and conducting various treatment studies seem to be essential. GBM-contracted dogs are one of the suggested models for treatment studies [5]. As morphologic and histopathologic characteristics of GBM in dogs are in many aspects similar to human GBM, some researchers hope that they, by curing brain tumors in dogs, could find a treatment model for human GBM [6]. However, doing experimental researches on cancer-contracted dogs has some limitations due to observance of animal rights and protection rules and ethical principles of empirical experiments on domestic animals; and using this animal is only then allowable that the suggested treatment has passed all experimental studies successfully. Just before applying this new method of treatment in human clinical phase, the cancer-contracted dog must be treated with it [7].

Athymic nude mice were introduced in 1968 by Pantelouris [8]. These mice do not have the thymus cortex, so their T-lymphocytes haven’t become mature and they lack cell immunity. One year after the introduction of these mice, the first xenograft model of tumors was created [9]. Recognized associations like the Us National Cancer Institute, have been using the xenograft models in their cancer studies and they conduct their preclinical phase of anti-cancer drugs on these models [10, 11, 12], as these models, due to use of human cancer cells, enjoy more validity comparing to other models [13].

Regarding the results of this study, the standard cell lines of GBM showed the same histopathological characteristics as human GBM and the new tumor graft to be transferred to mouse indicated the same characteristics as patient’s tumor. Researchers have suggested various treatment methods for GBM, however, to date, none of these offered methods, except for a few new ones, have reached the human clinical phase. Anyway, there is the hope that longer survival time can be achieved for GBM-contracted patients by continuing these studies. To date, limited in vitro pharmacological and radiotherapeutic studies have been conducted in Iran on the GBM cell lines [14, 15] but none of them have been assessed by in vivo tests due to the lack of animal models. Development of GBM xenograft models simultaneously from both standard cell line and human tumoral graft to be transferred directly to nude mice, is achieved in Iran for the first time and we hope that the investigators take positive steps in basic studies of central nervous system (CNS) primary tumors by using these models and can also determine the pharmacogenomic differences in Iranian races by evaluation of standard chemotherapeutic drugs used for both standard and autochthonous cell lines. We hope that these models make the evaluation of new anti-cancer drugs introduced for the treatment of this disease possible. At present

![Figure 2:](image)
a) the scene of pseudopalisading necrosis, in which the necrotic region in the middle is surrounded by tumoral cells. This is one of the most important diagnostic signs of GBM (H&E). b) Immunohistochemical staining using GFAP. c) Immunohistochemical staining using Ki-67.
anti-angiogenic drugs are being suggested for the treatment of this disease and Bevacizumab is being prescribed to human patients but, due to its side effects, there is disagreement over its efficiency to increase the patients’ survival time [16]. Now Iranian investigators will be able to evaluate the effectiveness of many drugs, which are expected to inhibit the angiogenesis of GBM tumors, through these models.
REFERENCES


