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Advanced Views of Glioblastoma Multiforme U-87 Cells for Therapy of Brain Tumor

Kaja Urbańska[1,*], Chandi C. Mandal[2,*]

[1]Division of Histology and Embryology, Department of Morphological Sciences,
Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW
Nowoursynowska 159, 02-776 Warsaw, Poland
[2]Department of Biochemistry, School of Life Sciences, Central University of Rajasthan
NH 8, Bandarsindri, Kishangarh, Ajmer-305801, Rajasthan, India

[*]Corresponding Authors:
E-mail - ccmandal@curaj.ac.in, kaja.urbanska@onet.eu

Abstract: *Glioblastoma multiforme (GBM) is the most common primary tumor of a central nervous system, with the IV grade of histological malignancy. Despite the advancement of GBM treatment strategies the overall survival in GBM patients is still less than one year and it is probably due to location of the tumor and its high proliferative as well as metastasis activity. Thus, there is an urgent need to intensify basic as well as clinical research on GBM for understanding the molecular biology of the diseases and for the treatment of brain tumor. Most of therapeutic and molecular studies are performed with using of commercially available GBM cell lines. Use of the right cell line, which is based on the precise knowledge of its characteristics, may not only facilitate to design the experiments but it also provides more authentic information from the study. Thus, the aim of this study is to present the characteristics of the U-87*glioblastoma *cell line, which is the most frequently used in brain tumor research. In the presented article there are the information indicating the similarities in U-87 cells and spontaneous GBM biology, description of current studies with using this cell line, as well as benefits of choosing U-87 cell line in researches.*

Keywords: *U-87*glioblastoma *cell line; Brain tumor; Glioblastoma multiforme; Malignancy.*

Introduction

Most of oncological studies related to glioblastoma multiforme (GBM), especially at the preclinical stage, are performed with using of established and well characterized tumor cell lines. Tumor cell lines can be obtained from cell lines banks: ATCC (*American Type Culture Collection*) or ECACC (*European Collection of Cell Cultures*), which have the largest collection of cell lines of human and animal origins.

U-87, U-251, U-118 and T-98G are commercially available human GBM cell lines [1] which are often used in GBM research. To enrich the bank collection of these types of cell lines, new human GBM cell lines have also been established such as WJ1 line [2]. There are several GBM cell lines available, which are derived from animals such as CNS-1 and C6 from rat and GL261 from mice, but these are chemical induced glial tumors, not spontaneous. Alternatively, the primary cell lines isolated from the tumors of GBM patients taken during surgery can also be used.

U-87 cell line is the most regularly used in neuro-oncology researches [3]. It has been analyzed in at least 1,700 publications [4], both *in vitro* (monolayer and spheroids) and in *vivo* and also *in ovo* experiments [5-8]. Thus, the aim of this study is to summarize the basic characteristics of U-87 glioblastoma cells and its various applications in neuro-oncological studies.

Characteristics of U-87 cell line

U-87 MG cell line was derived from Caucasian male tumor resected intraoperatively and characterized by Ponten et al in 1975. U-87 MG cell line is a heterogeneous line which contains two cell-types: adherent cells and small sphere cells forming aggregates [9]. Basic information of U-87 cell line is summarized in Table 1. Recent study has shown that this cell line has a highly aberrant genomic structure [4]. U-87 is a hypodiploid cell line, with the modal chromosome number of 44 occurring in 48% of cells with a 5.9% rate of higher ploidy [10]. The systematic, thorough, and accurate mutational analysis of the U-87 genome comprehensively have identified different classes of genetic mutations including single-nucleotide variations (SNVs), insertions/deletions (indels), and translocations. It was found 2,384,470 SNVs, 191,743 small indels, and 1,314 large structural variations. Mutational analysis revealed 512 genes homozygously mutated, including 154 by SNVs, 178 by small indels, 145 by large microdeletions, and up to 35 by interchromosomal translocations [4]. Similar as in spontaneous glioblastomas, there are many mutations in U-87 cells genes such as deletion of *p14*^{ARF} and *p16*. U 87 cells synthesize mutant form of tumor suppressor PTEN protein which in turn activates PI3K/Akt, which play a key role in proliferation, angiogenesis and resistance to the apoptosis [1]. U-87 cell line expresses a wild-type p53, tumor suppressor protein [11].

Table 1: Basic information of U-87 cell line – summary

U-87 cell line	
Derived from	Human, male
Disease	Glioblastoma multiforme
Karyotype	Hypodiploid
Growth medium	EMEM/DMEM*
Growth properties/morphology	Adherent, epithelial
GFAP expression	-/+*
S100 expression	-/+**
Ki-67 expression	+
Vimentin expression	+
Wild-type p53	+
Mutant p53	-
Ability to grow *in vitro*	+
in ovo	+
in vivo	+***
Ability to form spheroids	+

*****conflicting data; ******depending on culture model;
*******immunosuppressed and nude mice /rats

U-87 cells are negative for glial fibrillary acidic protein (GFAP) [1] - intracytoplasmic astrocyte-specific intermediate filament protein, the most specific marker for cells of astrocytic origin under normal and pathological conditions [12]. However many investigators have shown that low expression of this protein can be observed in U-87 cells cultured *in vitro* [6, 13-14]. Low or lack GFAP expression correlates with increasing grade of astrocytoma and there is also a progressive loss of GFAP expression in tumor cells of GBM [15]. U-87 xenograft tumors are GFAP negative. This does not necessarily mean that U-87 cells are of non-glial origin, but the ability to synthesize GFAP after further dedifferentiation of the U-87 cells in the host microenvironment is gradually decreased [6,13]. But S100 (acidic protein of glial cells) immunoreactivity is detected in cell suspension and in spheroids of U87 cells. It was reported that U-87 tumors developed in animal are S100 negative, which can be explained by the possible down-regulation of S100 expression following dedifferentiation of tumor cells [6].

However, considerable variations in the expression of S100 are also observed in human brain tumors, as well as within histologic types [16].

However, the experimental growth conditions significantly influence the gene and protein expression profiles of U-87 cells. It has been documented that the gene expression patterns are not the same when U-87 cells are cultured in *in vitro* and *in vivo* systems. Moreover the gene expression profiles differ between the subcutaneous (s.c.) and orthotropic *in vivo* growth conditions. Similarly, U-87 cells grown in intracerebral mainly express the genes related to central nervous system functions as compared to expression of cell cycle progression and regulation genes. These data suggest that the tumor microenvironment definitely has a profound effect on the gene expression of U-87 cells. Moreover, it may be that under *in vitro* conditions, the genotype of the cell plays a major role in determining the genes expressed [17]. These data indicate that different growth condition and microenvironment of the tumor cells may alter DNA methylation to do epigenetic regulation of many genes such as GFAP [14].

U-87 stem cells

U-87 MG cell line contains tumor neural stem cells, which express CD133 antigen, similar as in spontaneous GBM. This subpopulation of tumor cells possesses the ability of self-renewal and multipotency. There are available effective methods for isolation of tumor stem cells population from U-87 cell in literature [9, 18]. This fraction of U-87 cells is resistant to Fas-induced apoptosis, which could be implicated in the resistance of GBM to conventional anti-cancer drugs (such as doxorubicin, etoposide, carboplatin) and explain the relapse occurring after treatment. GBM stem cells are also less sensitive to radiation [19]. Thus, U-87 cell line can be used to evaluate the sensitivity of tumor stem cells to new molecules in therapeutic research pathways [9].

U-87 cell line in *in vitro* studies

Many research works have used U-87 cell line to conduct *in vitro* experiments. It is most frequently used to test the cytotoxic activity of antitumor agents (including nanoparticles and plant extracts) with demonstrating a generalizable role of proteins involved in GBM cells proliferation, migration, invasion, chemoresistance or radioresistance [20-24].

The invasive potential of U-87 cells is higher as compared to other GBM cell lines such as U-118. It results from the ability of U-87 cells to highly or exclusively expression of proteins which include ADAM9, ADAM10, cathepsin B, cathepsin L1, osteopontin, neuropilin-1, semaphorin-7A, suprabasin and chitinase-3-like protein 1. U-87 cells also express low levels of some cell adhesion proteins such as periostin and EMILIN-1 [25]. Studies of the molecular mechanism of invasion introduce the targets for glioblastoma treatment and can also identify the factors which activate metalloproteinases secretion and promote GBM invasive potential [26]. U-87 cells have a mesenchymal gene expression profile similar to that of primary glioblastomas [27]. It is also reported that U-87 cells, similar to spontaneous GBM are positive for vimentin [6]. Thus, U-87 cell line is also used in the studies of epithelial to mesenchymal transition (EMT) process. The EMT is defined as the occurrence of a variable proportion of tumor cells, which upregulates mesenchymal markers such as vimentin and snail, and that downregulates epithelial markers such as E-cadherin. This process promotes the emergence of tumor cells with mesenchymal traits essential for tumor invasion/metastasis and the self-renewal properties but reversal of EMT is needed for colonization of tumor cells in metastatic sites. EMT also helps to maintain cancer stem cells [28]. Tumor microenvironment/hormone/ other factors might play distinct role in regulation of EMT of cancer cells as well as reversal of EMT in different sites and it could be due to the effect of epigenetic gene switching on/off.

U-87 cell line in 3D cultures (spheroids of U-87 cells)

Tumor cells cultured in two-dimensional monolayer do not always substitute *in vivo* environment properly. Monolayer culture consists of cells generally growing in a nutrient enriched environment with adequate supplement of oxygen, which forms a nearly homogeneous colony. Cells of anchorage dependent culture lack the architectural and cellular complexities which are present in tumors, which include inflammatory cells, vasculature, and other stromal components. An *in vivo* mature tumor with an extensive vasculature has a very complex structure, generally consisting of regions of regularly dividing cells, hypoxic cells, and necrosis, at increasing distances from blood vessels with a three-dimensional (3-D) pattern bearing structural heterogeneity [29-30]. Due to these facts, *in vitro* cultures do not show the complex interactions that exist between the tumor and its host, so better solution could be spheroid culture which may more mimic to tumor structure. Moreover, multicellular tumor spheroids more resemble to tumor microenvironment and 3D organization as currently observed in avascular tumors [31]. These model systems can be utilized to test, chemosensitivity of cancer cells against a particular drug and to determine the impact of infiltrated cells on tumor growth and metastasis. U-87 cells usually generate tight spheroids, with excellent reproducibility and the Gaussian distribution of the spheroid volume is maintained 14-days. Such long period of spheroid culture could be ideally utilized to perform growth kinetic assay. There are established and validated a suite of highly reproducible U-87 spheroids microplate three-dimensional functional assays to enhance the biological relevance of early preclinical cancer studies. These assays will increase the translational predictive value of *in vitro* drug evaluation studies and reduce the need for *in vivo* studies by more effective triaging of compounds [32]. Results of studies performed on U-87 spheroids also depict what therapeutic strategies should be applied to treat GBM patients [33]. It has been reported that irradiation can enhance the cytotoxic effect of temozolomide (TZM), but the exerted effect is less than additive in terms of spheroid growth and migrational behavior [34]. Other results have demonstrated that the cytotoxic effect of the alkylating drug, such TZM, is manifested by increase of apoptosis process as well as intensive senescence-associated galactosidase activity in U-87 spheroids. Thus, these results indicate, that in the GBM treatment might be important to apply TZM at the maximal dose tolerated in the first cycle in order to avoid selection of resistant cells [33].

U-87 cell line xenograft models (*in vivo* and *in ovo*)

U-87 cells can be implanted on the chicken embryo chorioallantoic membrane (CAM) and into the rodents (mice or rats) brain. The suspension of U-87 cells (3-5×10^6) should be inoculated underneath of CAM. Such concentration is sufficient to observe tumor growth after two days of transplantation [6]. In support to these observations our research work show the cell morphology of U-87 cells when it is cultured in *in vitro* and tumor growth in chicken embryo (*in ovo* model) (Figure 1-3). Intracranially implantation of at least 1 x 10^6 U-87 cells to the athymic mouse leads to tumor growth [5]. U-87 cells in xenograft tumor have fast and aggressive growth [35]. The expression of tumor progression genes (IL-6, IL-8 and cysteine-rich angiogenic inducer 61), tumor regulators (lumican, F-box-only 6) and other cytokines in U-87 cells transplanted to the athymic mice has similar pattern as in spontaneous GBM. In addition, monocyte chemotactic protein-1 (MCP-1) which is elevated in GBM patients, is also highly elevated in U-87 cells [36-37] and elevated MCP-1 helps to recruit other cells such as monocyte/macrophage to tumors. Moreover, U-87 cells show similar protein expression profiles (related to Ki-67, vimentin, CD68, cathepsin B and cathepsin L and VEGF) in case of both *in vivo* and *in ovo* model [6]. Despite high rate of U-87 cell growth on *in vivo* and *in ovo* conditions, such tumors do not display several histopathological features of human GBM [5-6, 36].

U-87 *in vivo* models are used to identify the molecular and genetic regulation of GBM cell infiltration [6, 35]. It also has a potential use in testing new

drugs [38] and individual therapy [6]. U-87 tumors exhibit somewhat limited dispersal (only along blood vessels), they may be good models to analyze effects of chemotherapeutics on reduction of tumor load or migration on blood vessels. Moreover, human tumor-derived U-87 cells are highly angiogenic [35]. Thus the U-87 model has been widely applied and proved in assessing GBM angiogenesis [5, 39].

Future perspectives of U-87 cell line in oncological studies

Genetic manipulation of the U-87 cells (transfection, gene silencing) leads to characterize the proteins, which are beneficial for tumor cell growth and thereby identifies the possible targets for therapy [40].

U-87 *in vivo* model is simple and well-defined model of fast-growing GBM, which provide a basis for further experimental studies of genetic and protein expression fingerprints during human GBM tumorigenesis[13]. We have recently documented that the biological activity (defined as proliferative and apoptotic indices) of U-87 tumor growing *in ovo* is similar to the spontaneous GBM, so it seems, this model is also a good alternative for *in vitro* studies [41]. The only factor limiting the importance of the using *in ovo* model in GBM studies are the differences in bird's (recipient of tumor cells) and mammalian's metabolism (the donor of the cells). It refers to the phenomenon of drug resistance: birds are resistant to Chlormethine [42].

Despite the advantages of *in vivo* and *in ovo* models, these models still arouse some ethical controversy.

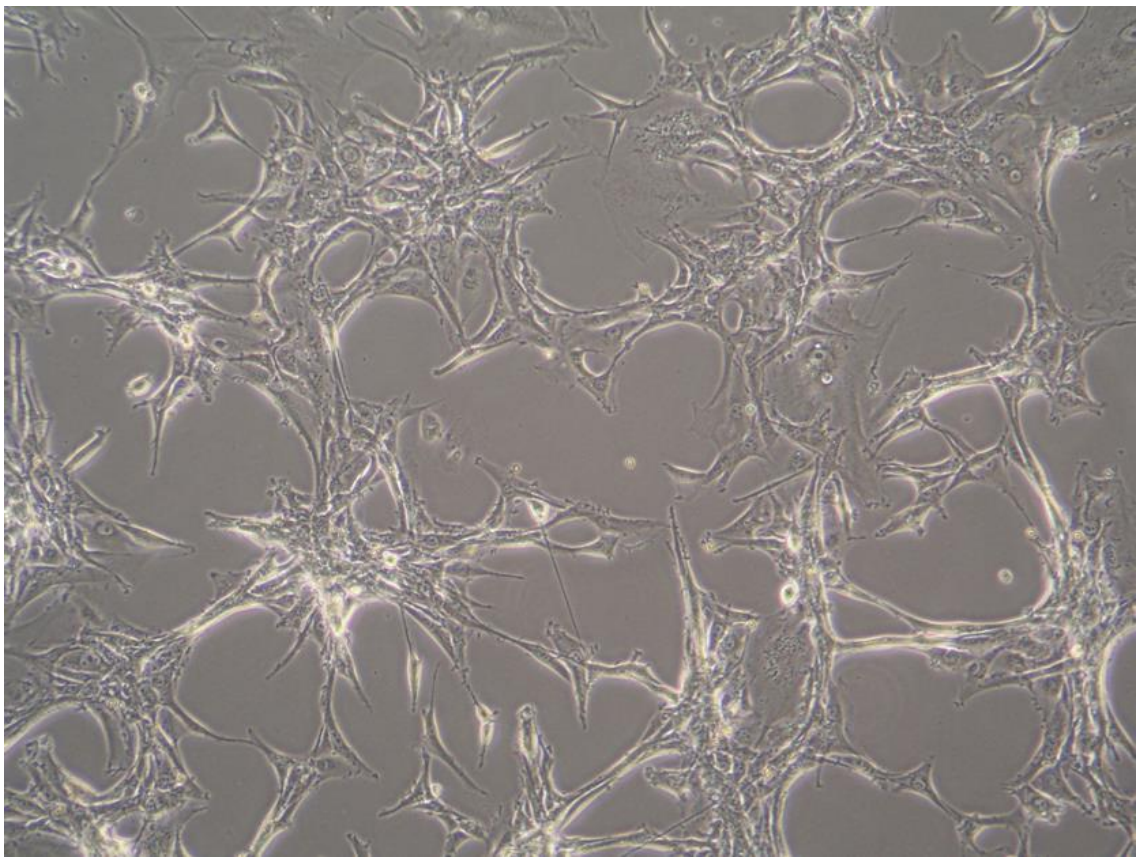

Figure 1. Morphology of U-87 cells - *in vitro* culture.
Cells were cultured under standard conditions (37˚C, 5% CO_2) in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with foetal bovine serum (FBS) and antibiotics.

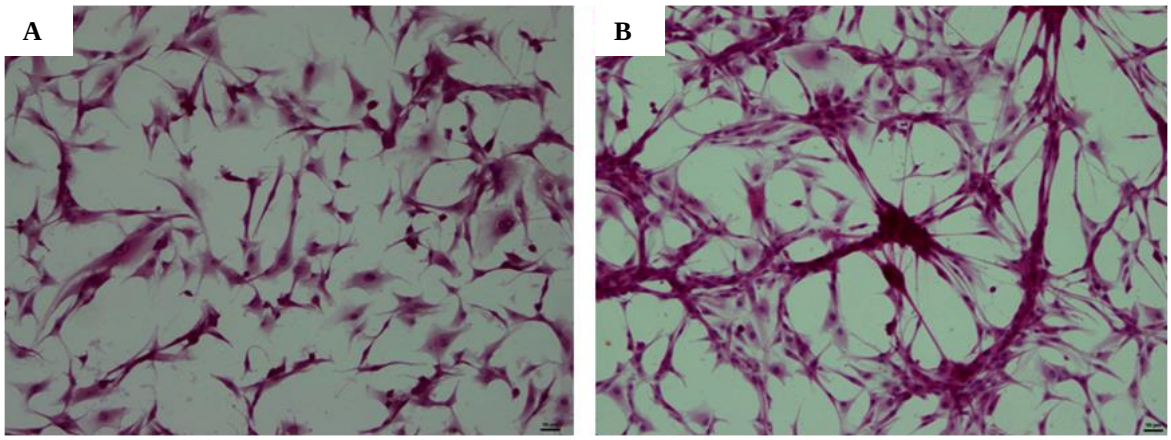

Figure 2. Haematoxylin and eosin (H&E) staining of U-87 cells;
(A) After 24 hours of culture and (B) After 120 hours of culture.

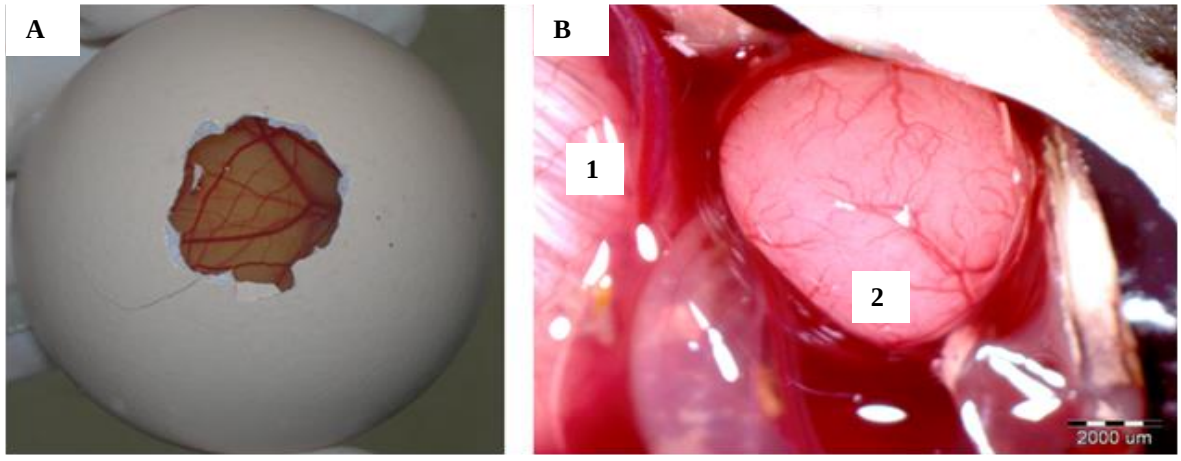

Figure 3. Human U87 tumor growth in chicken embryo.
(A) Chicken embryo chorioallantoic membrane (CAM) before U-87 cells implantation.
(B) U-87 tumor on CAM: 1 depicts chicken embryo; 2 depicts tumor.

Conclusions

Precise knowledge of the cell lines characteristics is fundamental during planning experiment and setting methodology by researchers and clinicians. The information about the properties of U-87 cell line allows to design more effective experiments and lead to carry out the researches about GBM at the cellular and molecular level with a greater ability. This cell line can be explored to determine the effect of drug on epigenetic gene regulation since gene expression profile markedly varies in different microenvironments and in different growth conditions.

Conflict of Interest: Authors have no any conflict of interest.

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