# Classification and Application of the Personalized Glioblastoma Organoids

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**Abstract.** The severe survival rate of glioblastoma especially the recurrent glioblastoma, has directly shown the aggressiveness of glioblastoma and meanwhile, showing the lack of the standardized therapies can be effectively and directly against the glioblatoma in clinical situations. The tumor organoids technology has developed rapidly and the personalized glioblastoma organoids have already been used in several researches about the therapies of glioblastoma as an appropriate model. Comparing with the existed models used in past researches, the tumor organoids show irreplaceable features which will contribute a lot in the researches of glioblastoma including the recapitulation of the microenvironment and the cellular communication of parental tumors. In some researches, personalized tumor organoids were used in drug-screening tests and the final-result show high similarity to the actual situations. The drawbacks of common models greatly hamper the development of novel therapies of glioblastoma because of its inaccuracy in the aspects of the recapitulation and experiment results which will prove a invalid therapy in clinical situations as effective and finally deviate the researches from the correct way. Personalized glioblastoma organoids can be divided into 2 types which is the 3D bioprinting organoids and the patient-derived glioblastoma organoids and each of these maintains own advantages in practical situations. Furthermore, personalized glioblastoma organoids can be stored for much more longer time than the existed organoids which also show the possibilities of applying the glioblastoma organoids into the biobank in order to assist further researches.

**Keywords:** Personalized glioblastoma organoids; Biobank; Tumor.

## 1. Introduction

The glioblastoma is characterized by the aggressiveness and it's also the most common primary malignant tumor of center nervous system in adults [1].The aggressiveness of the glioblastoma is mainly caused by its relapse after applying chemotherapy, radiotherapy, immunotherapy or surgery which may highly correlated with the glioblastoma stem cells and the high intertumoral and intratumoral heterogeneity [2]. In clinical situations, the difference between the individuals firstly diagnosed with glioblastoma and the patients with the tumor recurrence is especially conspicuous. Newly diagnosed patients usually maintain satisfactory treatment outcomes after the surgery followed by the chemo and radiotherapy, however, even though those therapies were proved to be effective in prolonging patients' lives, the possibilities of tumor recurrence or even death after first-line or second-line therapy [3], which account for a larger proportion of all the patients, which can be reflect in the investigation of the survival outcomes. The median overall survival after first-line surgery is approximately twice about that of second-line surgery [4].The decreasing of the median survival after first therapy progression compared with the counterpart after second-line surgery, also indicates the aggressiveness and refractory property of the recurrent glioblastoma.

Tumor organoids technology has developed rapidly during the past few decades. Personalized tumor organoids can be divided into 2 types which is the 3D bioprinting organoids and the patient-derived organoids. There are advantages to each of both.3D bioprintng is characterized by its precision which means high degree of similarity to the parental tumor, but it still means high cost and demanding biomaterials such as a the printable 'bioink'. The patient-derived organoids also resemble the characteristics of the original tumor including genotype, phenotype, invasiveness, heterogeneity and cellular communications [5]. Using the organoids technology during the cancer treatment, not only the more accurate drug response related data will be available for further treatment, more clinical

appliances will also be exploitable such as creating living biobank of certain refractory tumor organoids which can be used as a pre-screening of cancer therapy and sharing the available data with the global institution.

The main reason of the unsatisfactory prognosis in the clinical recurrence cases is that, first of all, few attention has been paid into the cellular and molecular heterogeneity which will be different between tumors of individuals and more complicated in the recurrent tumors in the exsiting therapy and secondly, none standard clinical therapy has been clearly defined as the rule therapy which was directly against the recurrent glioblastoma [6].In the treating progress of the recurrent tumor, the treatment decision often need to be done after multi-disciplinary discussion based on the actual situations which greatly increase the difficulty of healing the recurrent tumors. Also existing research has shown that post-surgical treatment may cause the plasticity of the tumor cells which will induce the more aggressive phenotype [7], treatment resistance and finally cause the opposite consequence. In current brain tumor therapy, several challenges still existed which hampered the development of the novel treatment. In the clinical situations, sensitivities of different individuals with the same tumor varied greatly to the same common cancer therapy caused by the individual difference which can also show the importance of the personalized cancer therapy because in personalized treatment, more attention will be paid into the aspects which existing treatment didn't attach much importance to reckon with, in other words, personalized treatment may greatly erase the side effect brought by ignoring the individual difference and finally surmount the barriers of the poor prognosis. Personalized tumor organoids is one specific way to realize the individualization of the cancer therapy because the personalized tumor organoids is the 3D culture of the tumor cells which maintains similar characteristics to the original tumors [8].Organoid technology in brain cancer also maintains significant advantages compared with the common models used in the past experiments such as cell lines, patient-derived xenografts and tumor spheroid because it enables conservation of cellular interactions and also maintains heterogeneity of the original tumorwhich is also the key reason why the common models without these characteristics hamper the development of the novel therapy and medicines [9].

Because of all the aspects mentioned above, individualized therapy which obtains the ability to treat each individuals precisely will be the essential aspects of cancer therapy in future. This dose not imply that current therapies commonly used are totally nonsense but to promote the refinement of the therapies and strive for higher survival rate. This review will focus on the classification and the application of the personalized glioblastoma organoids and the certain advantages of applying organoids technology will also be included in.

## 2. Organization of the Text

### 2.1. The comparing between the common tumor models and personalized glioblastoma organoids.

#### 2.1.1. The common tumor models of glioblastoma

Novel cancer treatments such as the tumor vaccine, immunotherapy, targeted therapy, have been developed during years of researching. Especially, the immunotherapy has been awarded the Nobel Prize in medicine in 2018 which also reflected the importance of the immunotherapy and the huge progress has been made in the aspects of overcoming cancer [10]. Despite the endless stream of the innovations in cancer therapy, still few new treatments have proven to be effective through testing on the common tumor models currently make it passed the approval by the Food and Drug Administration (FDA) and even when they do, most of those treatments still maintains limited success rate and limitations on usage in clinical situations. For example, the immunotherapy for brain cancer often lose its theoretical effect because of the brain-blood barrier which will continues prevent the invasion of anti-tumor drugs [11], resulting in unsatisfactory treating outcomes.

The reason why most of the therapy proved to be effective during repeated experiments but still be fail to achieve desired results in practical clinical applications is the lack of the more appropriate and more accurate model which can precisely imitate the similar conditions of the human in vivo environment and show the therapy targets promptly during experimental phase than the common models such as cell lines, patient-derived exnografts and tumor organotypic explant. It's the same when comes to glioblastoma. These models got their own merits without doubts, however, several shortcomings existed need to be improved.

### 2.1.2. Glioblastoma cell lines

The human glioblastoma immortalized cell lines commonly applied in practical situations are the U87MG, U252, T98G and LN-229 [5]. These cell lines can provide the certain glioma cells ultimately which don't need to worry about the problem caused by the ethical concerns which also significantly increased the speed of obtaining the preliminary result of new anti-tumor drug vitro testing [5].The human glioblastoma cell lines also represent the basic features of glioblastoma which provide a method to investigate the glioblastoma biology. For example, by observing the expression of stem cell markers in the glioblastoma cell line-derived sphere grown in the serum-containing medium and the same counterpart grown in serum-free medium, glioblastoma cell lines show that higher level of aggregation of the cancer stem cells will appear when this kind of sphere grow in the serum contained medium [12].However the lack of long-term stability which is specifically characterized by genetic and transcriptional mutations caused by the rapid cell proliefration and the in vitro cultures-imposed selection [5] which will cause the unreliability and difficulties in further research. It's also hard for cell lines to show the exact cellular communications and mechanism during tumor development because of its 2 dimensional structure. These drawbacks explained that why cell lines were used for obtaining approximate and preliminary data.

### 2.1.3. Patient-derived xenografts (PDX)

PDX is the model which transplant the biopsis or disassociated tumor cells derived from the patient into the immunosuppressed rodents which can be classified into heterotopic xenografts and orthotopic xenografts. Rodents commonly used in the PDX experiments can be classified into 3 types, the mice are unable to produce T cells, the mice are unable to produce T cells and B cells and the mice are unable to produce T, B and NK cells. The advantage of PDX is that it won't change neither the phenotype nor the heterogeneity of the parental tumor [13].The genetic stability will provide great convenience to further experiments compared with the cell lines.

Also, PDX provide central nervous system microenvironment which act as an essential aspect in the development of glioblastoma [5].Because of the microenvironment established in PDX which is close to the original in vivo environment of the glioblastoma, PDX has been broadly applied into the current experiments. Features mentioned above explained the reason why PDX can be used as a carrier of individualized drug-screening and the drug resistance mechanism will also be observed meanwhile [5].However, the PDX still maintains several challenges impose restrictions on its usage such as the difficulty of technology, high cost and prolonged latency which were caused by the specific difference between human and rodents including the aspects of cellular and molecular levels [9].

### 2.1.4. Glioblastoma organotypic explant

The glioblastoma organotypic explant can be established through in vitro tumor cells transplantation into brain slice which provide the natural and organotypic environment of the glioblastoma including blood vessels, immune cells and neural cells. The organotypic cultures open the possibility of genetic engineering on both tumor cells and brain microenvironment [14].Several researches have been done during past few years. Research have transplanted the glioblastoma cells in the human brain slice in order to investigate the interactions between glioblastoma cells and host cells in 2018 [15].Another research have applied this system into evaluate the radio response and radiosensitizers in 2018 [16].

Initially, this model was applied into the research about the tumor cell invasion in the normal brain environment [17]. After years of development, the current over all application of the glioblastoma organotypic explant is the investigation of the glioblastoma biology including the interaction between tumor cells and tumor microenvironment, and evaluation of the drug response. This model also shows drawbacks such as lack of long-term stability and expandability, high degree of technical difficulty during manipulation which put restriction on the application of this model [9].

### 2.1.5. Tumor organoids models of glioblastoma

The realization of both of the tumor organoids and common models of tumor are based on the high degree of the proliferation of the tumor cells and from the cost aspect, common models are more financially user friendly which seemed to imply that tumor organoids actually has limited application. However, the several drawbacks of the common models always cause inaccuracy of the test result, on the other hand, glioblastoma organoids which were aimed at recapitulating the glioblastoma [14]can make up for deficiencies and the overall advantages of it including the ability to maintain long-term stability, high level of precision and avoiding other shortcomings common models have shown [9].At the same time, the growth of tumor organoids is not totally perfect. Compared with the xenografts or tumorspheres, the growth of tumor organoids still maintains the shortcomings such as the time consuming and varied cellular growth rate according to the region of tumor [18] which needs to be further improved before practical application.

The way to realize the tumor organoids are varied. Hubert et al. established the ex vivo model which can establish the brain tumor organoids through disassociating the tumor samples into the single cells including stem cells and non-stem glioblstoma cells [18].Other ways such as culturing the tumor biopsis directly also proved to be effective, for example, research team cultured the patient-derived glioblastoma organoids directly from the tumor tissue of the patients. Also, the techniques used during culturing an tumor organoid is demanded differently depending on the aim of the research. Researchers have used the organoid culture which didn't maintain the EGF, bFGF, serum and extracellular matrix in order to avoiding the single-cell disassociation and make the organoid finally maintain high degree of similarity to the parental tumors [13].Another research team cultured the organoids in the neurobasal medium which maintianed the ability to support the growth and functional recovery of the nerve cells, supported with EGF,bFGF,B27,glutamine,antibiotics and sodium pyruvate in order to guarantee that tumor cells will grow in normal way and gain the better outcomes [18].

In the past decades, organoids technology has developed rapidly and tumor organoids will be mentioned will be classified into the 3D bioprinting glioblastoma organoids and patient-derived glioblastoma organoids according to the specific way of establishment.

### 2.1.6. 3D bioprinting glioblastoma organoids

The 3D bioprinting glioblastoma organoids are realized through a fixed way which deposit layers of biomaterials precisely and finally recapitulate the exact extracellular matrix of the parental tumor [5].As shown by the mechanism,3D bioprinting technique is characterized by the accuracy which include the similar heterogeneity of the parental tumors and avoiding unwanted genetic mutations during culturing which are the key points that research team of normal tumor organoids strove to achieve. Compared with the common 2D glioblastoma models,3D bioprinting organoids not only maintain the advantages which commonly owned by the tumor organoids including the show of tumor associated microenvironment, cellular interactions and in vivo tumor origin,but also show the feature of precision [9].In the specific application in glioblastoma, the 3D bioprinting organoids will provide more accurate and personalized data derived from the tumor which can highly promote the development of individualization of cancer therapy. Researchers have explored the 3D bioprinting of the glioblastoma stem cells which show positive results and they evaluate the drug tolerance of the 3D bioprinting organoids which was higher than the parental tumor especially to the cisplatin which has failed in many clinical situations [19].Because the result achieved by them was close to the real

situation so the 3D bioprinting glioblastoma organoids represented the feasibility of applying it into the drug-screening test.

Many new technologies has been applied into the 3D bioprinting organoids in order to guarantee the accuracy of the final products such as the microfluidic technology and the computer controlled additive biofabrication used during bioprinting[9].Because of the precision feature and the reliability of the 3D bioprinting technology,3D bioprinting organoids can show the patient-specific data such as pivotal tissues or microstructures [5]which will increase the accuracy of the drug testing experiments and the prediction of the drug response, and further provide more accurate clinical diagnose and personalized medicine and also make huge contribution on the study of glioblastoma-associated macrophages[17].

However, the researches of 3D bioprinting have been limited because they mostly payed too much attention into the traditional biomaterials which do not be effective to represent the complexity of the in vivo microenvironment [20].In addition, the 3D bioprinting of the human sized organs hasn't been realized to date which implies difficulties during the realization of the 3D bioprinting glioblastoma organoids and the lack of the appropriate printable bioink is also the main restriction during 3D bioprinting, the stability of the bioink during printing and transportation are also essential to the practical application [20].In order to take over the existed challenges about bioink, several researches have been done. Researchers have devoted themselves for years to explore the extracellular matrix(ECM) derived bioinks which contains key components boost viability of tumor cells and phenotype [20].Other research team have developed a mechanically robust thixotropic collagen hyaluronic acid bioink which was able to establish self-supporting structure with the absence of the sacrificial supporting materials [21].

The limitations also mean the opportunity of further development and the 3D bioprinting glioblastoma organoids will be further developed and applied into more clinical trials after those drawbacks are overcome.

### 2.1.7. Patient-derived glioblastoma organoids(PD-GBOs)

PD-GBOs are also the 3 dimensional model which clearly maintains cellular and molecular interactions and also, the morphological features were included in this model [1]. Researchers have proved that the PD-GBOs can also recapitulate the actual glioblastoma characteristics inculding the high level of proliferation of the tumor cells, the expression of the glial fibrillary acidic protein (GFAP) which highly expressed in the glioblastoma tumor cells and also play an important role in the aspect of maintaining neuronal structure in center nerve system, and the presence of the morphological features such as the tumor microtubes (TMs)which can not only promote the cellular interactions, but also facilitate the resistance of diverse therapies [1]. Because of the features of TMs which are shown to positively support the unlimited growth of the glioblastoma, TMs would better to present in the models or it's hard to guarantee the accuracy of drug testing. The research team observe the characteristic markers which expressed on TMs or at the contact sites of TMs, such as Gap43 and Connexin43 [1]. The research team explored that huge difference have been shown between individuals by doing drug-screening and calculating the z-scores [1] even though the four patients all have glioblastoma which show nothing different between each other from the diagnose before this experiment, however, PD-GBOs reveal the truth to everyone eventually.

The TME which common models are generally unable to represent, however, greatly emphasized during researches. The reason why TME is so important that it will influence the accuracy and reliability of experiments is that metabolism which is also highly correlated with the tumor growth and metastasis can't be represented accurately without the existence of TME [9].PD-GBOs can greatly represent the TME which can provide the clue about the certain metabolic feature of glioblastoma which may explain why certain therapies can't be as effective as assumed through theory. In the research, they found the hypoxic core in middle of the PD-GBOs and the cancer stem cells in this region have slow cell cycle and rarely undergo apoptosis, in contrast, the cells in the layer of PD-GBOs cycled rapidly [18].The hypoxia always cause metabolic abnormality of lipid in order to avoid the damage of hypoxia and in the hypoxic region which maintains more stem cells, more

polyunsaturated fatty acid will appear which will be used to maintain the ability to realize the progression of self-renew [22].Combining with the result of the researchers, a feasible way to detect and suppress the stem cells inside glioblastoma can be realized.

The long-term stability of PD-GBOs also provide huge convenience to the researchers. Research team compared the genetic expression of PD-GBOs which have been separately cultured for 1, 2, 4 and 12 weeks with the counterpart of parental tumor and the result implied that PD-GBOs can maintain the high similarity to primary glioblastoma for more than 12 weeks [13]. The excellent stability of PD-GBOs will avoid many uncertainties during the experiments such as unwanted genetic mutations and the changes correlated with the heterogeneity or metastasis which will affect the result unconsciously. Also, in that research, they assumed to bank the PD-GBOs and developed the way of culture the organoids directly from the tumor biopsis which will be more stable than the way of disassociating tumor into single cells [13].Banking the PD-GBOs can be the solid foundation of creating the biobank or the platform with sharing data, which open the possibility to further explore the therapy to erase the side effect brought by individual difference.

### 2.2. The application of personalized gilioblastoma organoids

#### 2.2.1. The Glioblastoma organoids biobank

Biobank is the facility which maintains the capability to collect, store and manage the biologic samples such as specific tissues, cells and organoids and both of the data and samples stored in this bank can be used and even shared during experiments such as the research of the new target expressed on cells, drug-screening and exploring new therapy [13].Tumor organoids biobanks have been developed which included almost all the common cancer types, however, few glioblastoma organoids biobanks have been established [13]and the main reason include following points. First of all, in traditional researches, it was difficult to preserve or biobank the glioblastoma tissue for long-term, which was mainly caused by the low penetration of dimethly sulfoxide(DMSO)which played an essential role in preventing the damage from the tissue during the cryopreservation [23]. Secondly, the organoids, take the PD-GBOs as the example, need to be treated rapidly and carefully after being resected from the patients which means high demands on medical resource and meanwhile, the location of the laboratory shouldn't be far away from the hospital in order to keep the tissue fresh and avoid to use the cryopreservation and also increase the success rate [23].Other reasons such as the cauterization of the biopsis during the surgery and the changes happened after prolonged culture will affect the success rate of culturing and the accuracy of the recapitulation of organoids which increase more difficulties on biobanking glioblastoma organoids [23].

Even though, in French, a glioblastoma biobank existed which is able to analyze and share the data about the second-line therapies of glioblstoma currently used in clinical situations regardless of the exact effect of these therapies, this biobank contribute a lot in providing suggestions or evaluation results to the clinical second-line therapy [4]. However, obviously, the glioblastoma biobank still maintains several points out of reach, such as the lack of appropriate models can be used for evaluating the effect of certain therapy before applying it on the patient which show none benefit to promote individualized therapy. Also, less ethical problem will be caused because all the available data in glioblastoma organoids biobank is originating form the experiments on organoids. Additionally, living biobank of glioblastoma organoids will enrich the available resource for glioblastoma research [17].

Researchers have strove for establishing the biobank of glioblastoma organoids from multiple facets. Research team have cultured the biopsis with the specific inhibitor and developed a certain 3D tissue cryopreservation in order to decrease the damage caused by freezing [23].In addition, researcher established the living biobank which stored over 150 organoids and 40 patient-derived orthotopic xenografts(PDOX)models which also reflect the great developing prospects of the glioblasgtoma organoids biobank [24].

### 2.2.2. Personalzied glioblastoma organoids provide guidance of personalized drug therapies

In the treating progress of glioblastoma, especially the recurrent glioblastoma, it's hard to find a standardized therapy which can be commonly applied into most of the patients and finally achieve ideal therapeutic outcomes. Also, unexpected side effects may be aroused by using wrong therapies aimlessly which will worsen the current condition such as inducing the plasticity of tumor cells [7]. So, the selection of the glioblastoma therapy need to be carefully decided in order to avoid further damage to patients, however, given that only few experiential data with unsatisfactory outcomes can be learned from, it's more reliable to use the glioblastoma organoids to repeat the drug-screening experiments and through observing and analyzing the results, can the most ideal choice be explored.

Personalized 3D drug-screening can be applied to both the PD-GBOs and 3D bioprinting glioblastoma organoids. In the reseach, they found that cisplatin showed extreme unsatisfactory results on treating 3D bioprinting glioblastoma organoids which was close to the reality [19].In other research, the drug-screening tests have been applied in the PD-GBOs from 4 patients and the results of these 4 PD-GBOs show great difference [1].In the first two PD-GBOs, only crizotinib have been proved to be both effective to the first two PD-GBOs, and the afatinib and RXDX-101 proved to be effective to the third PD-GBO, however all of the 41 kinds of medicines which were approved by FDA showed invalid to the forth PD-GBO [1].The research also show the difficulty to confirm the certain therapy without drug-screening therapy. Both of the researches above imply the feasibility of using personalized organoids to provide guidance of personalized therapy.

### 2.3. The future prospects of personalized organoids.

The development of novel therapies were restricted because of the lack of the appropriate models. The further application of personalized organoids can be combined with the novel cancer therapies during the experimental period. Because of the accuracy of the 3D structure and TME recapitulated by the organoids, not only can the new therapy be found the unexpected limitations or shortcomings, but also, the more clear the mechanism and oncogenes will be learned through analyzing the organoids. The more targets can be aimed by chemo or immunotherapies will appear, further, when the technology have developed to be mature enough to explore the drug sensitive targets, it will be possible to develop the individualized therapy by patients.

## 3. Conclusion

Personalized glioblastoma organoids represent more biological features of glioblastoma and the individual difference has been clearly shown at the cellular level. Given that the researches of the targeted glioblastoma therapies require more accurate models, personalized tumor organoids may be developed more thoroughly and widely applied into the researches in the future. Especially the 3D bioprinting orgnanoids, which can be improved in several aspects. Combining the 3D bioprinting technology with other novel techniques including organotypic cultures or the calculation of artificial intelligence, the 3D bioprinting glioblastoma organoids will break more barriers in the way of current research and recapitulate the specific features more precisely. In addition, the glioblastoma biobanks will no longer be too demanding to realize as the development of 3D bioprinting technologies. The biobank of glioblastoma organoids will provide accessible data including the drug-screening data and structural data, which will contribute a lot during the further exploration of glioblastoma therapies. The organoids stored in the biobank can also be analyzed further and statistical results can be concluded which reveal the growth of both the glioblastoma organoids and the glioblastoma. The application of personalized gliobalstoma organoids mentioned above can be combined together. Applying drug tests in the glioblastoma organoids biobank will increase the accuracy of the drug-screening tests by utilizing the organoids models stored in biobank, meanwhile, the data base of the biobank will be enriched and finally more individualized therapies will be explored. Even though challenges still existed in the way of curing gliobalstoma, personalized glioblastoma organoids still can be developed as an irreplaceable tool in clinical research.

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