Potential use of glioblastoma tumorsphere: clinical credentialing

Seok-Gu Kang · Jae-Ho Cheong · Yong Min Huh · Eui Hyun Kim · Sun Ho Kim · Jong Hee Chang

Abstract A decade ago, cancer stem cells (CSCs) were introduced as target cells for an innovative cancer treatment. Particularly, there have been a lot of biological researches on glioblastoma (GBM) CSCs. However, as there is a comprehensive change in the concept of CSCs, it is required to review how the different CSCs for patients can be clinically used, or clinical credentialing, and summarize the possibilities of clinical credentialing. In this regard, this review aims to introduce the tumorsphere obtained from GBM specimen and summarize the clinical dilemma and clinically applicable areas.

Keywords Cancer stem cell · Clinical credentialing · Glioblastoma · Intratumoral heterogeneity · Testing platform · Tumorsphere

Introduction

Cancer stem cell and glioblastoma

Conceptually, cancer stem cells (CSCs) have both properties of the cancer cells and the stem cells (Sulman et al. 2008). In other words, CSCs have the tumorigenic property, which is one of the common characteristics of the cancer cells, as well as the self-renewal and differentiation property, which is the common characteristics of the stem cell. Since the hypothesis was published that the cancer cells with such stemness might be associated with treatment resistance (Donnenberg and Donnenberg 2005; Drewa et al. 2008), researches on this subject have explosively increased and the hypothesis has been recently proven (Duru et al. 2014; Perona et al. 2011).

Glioblastoma (GBM) is the most common primary brain tumor with an unfavorable prognosis that the median survival period is only 14.6 months despite the best treatments of surgery, chemotherapy and radiotherapy (Stupp et al. 2005). It was assumed that CSCs might be involved in such a bad prognosis and the existence of CSCs in the human brain tumors was first reported by Toronto group (Singh et al. 2003). After the report was released, there have been more in-depth researches on GBM CSCs (Piccirillo et al. 2009a, b). Among them are the researches on glioma and CSCs that the CSCs can be well isolated as the malignant grade of glioma is higher according to the World Health Organization (WHO) (Kong et al. 2013a, b) and that the biology of the CSCs change after serial transplantation (Shin et al. 2013).

Changing paradigm about the definition of classic CSC concept: tumorsphere

However, the term “CSC” has been less frequently used in recent years. One of the reasons is that the CSC hypothesis
that a CSC might be the original cell of “cancer organ” itself has been ambiguous. This was very fancy to explain the carcinogenesis process and treatment failure since the single CSC is a seed from the conceptual perspective of “cancer organ”. However, according to the research conducted by the Weinburg who was the leading exponent of the CSC theory, the cancer cell in a large tumor itself was reported to obtain or lose the property of stemness on its own (Chaffer et al. 2011). After this research, it was assumed that a CSC was not just a single original cancer cell but there would be a lot of CSCs in a tumor. Since then, the term “CSC” was not used much and, at the same time, there was a comprehensive change in the concept of CSC (Gupta et al. 2014).

Therefore, the cells with stemness property among the cancer cells were conceptually replaced by the comprehensive terms of “tumorsphere (TS)” and “sphere” since they were found in the cells growing in the form of sphere under the serum-free culture condition (Ledur et al. 2012). Moreover, as the cells with the classical property of CSCs are included in TS (Ahmad et al. 2014), we personally think that the term “tumorsphere” is currently more practical than “CSC”. However, the terms of “tumorsphere” and “CSC” or “cancer initiating cell (CIC)” are used as the same concept in many documents and theses (Romaguera-Ros et al. 2012; Sulman et al. 2008; Xu et al. 2008). In this review article, the term “CSC” or “CIC” or “TS” obtained from GBM, a conceptual definition that may cause confusion, was uniformly named and used as “GBM TS” (Fig. 1).

Clinical dilemma

Intratumoral heterogeneity of GBM

The term of ‘intratumoral heterogeneity’ is commonly used to describe the genetic heterogeneity of a single cancer mass from a single patient (Sottoriva et al. 2013). GBM is a tumor with representative intratumoral heterogeneity, which is assumed to be relevant to treatment resistance of GBM (Gerashchenko et al. 2013; Gill et al. 2014). According to the recent report, the tumor sampling at an interval of 1 cm for a GBM patient surgery showed different profiles of GBM in a single tumor at the transcriptional level and cancer evolution was found in time and space (Sottoriva et al. 2013). This intratumoral heterogeneity of GBM makes us to imagine that the tumor specimen where TS isolation was tried, will have different properties depending on the part of the tumor (Fig. 2). Our imagination brings a question on which part of the tumor should be obtained for GBM TS isolation. It is believed that the dilemma over which part of the GBM, the highly heterogenous cell population of GBM TS should be obtained from, can be an important experimental point on how to clinically use the isolated GBM TS in the future.

Different niches for TS in a same GBM specimens

Piccirillo et al. reported the differences in biological characteristics between the TS obtained from of the center of the GBM tissue and the TS obtained from periphery (Piccirillo et al. 2009a, b). The result that different types of TS are isolated depending on the part of the GBM mass where the TS is obtained from, is advanced as follows: The fluorescence-guided resection can be performed if the substance of 5-aminolevulinic acid is treated before the surgery of GBM (Stummer et al. 2000, 2006). It was reported that the TSs obtained from each area were different in terms of growth rate, stemness profile and tumorigenesis ability, when the biopsy was separately conducted depending on the fluorescence of GBM (Piccirillo et al. 2012). Therefore, the fact that the biological characteristics of the TS obtained from GBM is determined by the area where TS was isolated from is a research result that shakes the concept of “one seed, one cancer” under the classical CSC or CIC or TS theory, giving another dilemma to us over what is the genuine CSC or TIC or TS.

Non-tumorigenic stromal cells in GBM

In fact, there are some cells that change the biological characteristics of TS in the tumor microenvironment (TME) of GBM although it is confusing to think about GBM based only on TS. Among the GBM TME, the cells that are not independently tumorigenic but change the biological characteristics of the GBM TS have diverse names, commonly called as non-tumorigenic cells from GBM. The existence of the cells with mesenchymal property in GBM was first presented by Lang (M. D. Anderson Cancer Center) in 2007 ASCO meeting and 2008 SNO meeting (Lang et al. 2007, 2008) and the relevance of the cells with progression of GBM TS was presented in 2013 SNO meeting by Lang’s group (Hossain et al. 2011).

These non-tumorigenic cells were named to be glioma associated stem cells (GASC) (Bourkoula et al. 2013), tumor associated mesenchymal stromal cells (TAMSC) (Hossain et al. 2011), brain tumor derived-mesenchymal stem cell (BTM-MSC) (Behnan et al. 2014) and tumor mesenchymal stem like cells (tMSCLs) (Kim et al. 2011, 2013; Kwak et al. 2013; Lim et al. 2013). It is reported that GASC plays a role to predict the progression of the low grade glioma (Bourkoula et al. 2013), BTM-MSC is relevant to tumor progression (Behnan et al. 2014) and MSCL is associated with the increase in angiogenesis (Shin et al.
Fig. 1 One example of our GBM TS data set. When we isolated GBM TS, we characterized GBM TS by immunocytochemistry, 2D invasion assay, 3D invasion assay, q-PCR, and orthotopic xenografting.

Fig. 2 Magnetic resonance image and intraoperative photographs demonstrating different red fluorescence under violet-blue illumination. It showed different qualities of tumor fluorescence in a single tumor.
potential use of GBM TS

2013). As these TME cells relevant to TS provide more number of cases to find the origin of carcinogenesis using the GBM TS, more time and efforts are needed.

**Potential usage: clinical credentialing**

As testing platform

TS has played and will continue to play a key role to study biology of GBM and find the cause of carcinogenesis. Not many answers come to mind when we think about how GBM TS is clinically used and applied. However, there is a very important point about clinical applications. It is well known that the latest molecular target agents on GBM continue to fail to improve the overall survival (Chinot et al. 2014; Gilbert et al. 2014). These kinds of failure is because that there are too many therapeutic targets to increase the survival period of GBM patients using the latest targeted agents (Bai et al. 2011). However, one thing that cannot be overlooked is to reconsider whether a proper testing animal was used for the preclinical study and it reflected the real GBM of patients.

It is known that the existing commercial GBM cell line cannot mimic the real cancer cells of patients and those TS of GBM patients can show well the property of the existing tumors of the patients (Bjerkvig et al. 1990; Wang et al. 2009). Since there has been no research on the relevance of patient’s GBM and patient’s conditioned reprogrammed cells (CRC) (Liu et al. 2012), the desperate necessity can be expected at the in vitro level before making new attempts or giving treatment directly to the patients by using the TS isolated from the real GBM of patients. However, there is a problem that TS cannot be isolated from all GBM patients as the isolation rate of TS from GBM amounts to about 43.8% (Kong et al. 2013a, b). Therefore, it is considered that a higher isolation can be very relevantly applied to building the testing platform using TS in the future.

As GBM PDX model

The animal testing platform for cancer treatment and study was reported in 1968 by applying the cellular kinetic of combination chemotherapy to laboratory animals (Skipper 1968) and many more new therapies were tested at the animal level as a simple model of cancer cell line and xenografting. Hidalgo (Johns Hopkins Hospital) pointed out the inherent problem of such single cell xenografting and introduced the innovative method of tumor fragment in vivo transplantation (Rubio-Viqueira et al. 2006) rather than cell xenografting, which was the first concept of patient derived xenograft (PDX) that copies the original tumor of patients rather than cell xenografting. Since then, advanced cancer research institutes have been making efforts to make PDX core to theoretically immortalize the cancer fragments.

In terms of GBM, research results on testing platform that copies patients’ tumors were reported using the sphere (Wang et al. 2009) or dissociated cell (Joo et al. 2013). By applying the PDX model from a point of view to transplant the most original tumor fragment, the research result on xenograft that inserted the GBM fragments into the brain, which made it similar to the GBM of patients, was released in 2010 (Fei et al. 2010). Regardless of the methods, the key is the similarity to parental (patient) tumors. Therefore, it is regarded to save money and time for researches by making GBM PDX that is similar to mother tumors using GBM TS. TS was already reported as a concept of serial subtransplantation (Shin et al. 2013) and other items than molecular profiling were reported to have similarity to TS (Fei et al. 2010; Wang et al. 2009). Clinically, GBM TS will be a very valuable and important cell to secure similarity to original tumors of patients.

**Conclusion**

Still, many scientists are doing researches on GBM TS and new research results have been released on a daily basis. GBM is a very heterogeneous tumor so that the TS isolated from GBM is also heterogeneous. Although these heterogeneous TSs, have yet to be proven, it is required to verify whether they copy the mother tumors of GBM patients well through experiments. It is regarded that the GBM TS will become excellent source cells of patient mimicking testing platform in a clinical manner.

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**References**


