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Human Tissue Engineering: A Relevant Model for Identifying microRNAs Activated by Glioblastoma/Neural Tissue Interaction

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Abstract

A key challenge in glioblastoma biology and relevant tumor environment modeling lies in understanding of the cellular interactions occuring within the context of a 3-dimensional (3-D) human tissue microenvironment. Recently, human engineered neural tissue raise the opportunity to study such a very important feature of GBM *in vitro*. Here, we introduce this model for studying microRNAs.

Keywords: Tissue engineering; Glioblastoma; MicroRNAs

Human Tissue Engineering

Glioblastoma multiforme (GBM) is one of the most incurable forms of cancer in adults. Despite major research efforts and progress in neuroimaging, neurosurgery, chemotherapy and radiotherapy, the overall survival of patients with GBM has not dramatically changed over the past 30 years [1,2]. Today, surgery, chemotherapy with temozolomide and radiotherapy remain the standard of care in patients with GBM but the prognosis for GBM patients remains dismal with a median survival after diagnosis of about one year [1,2].

While the cellular and molecular mechanisms underlying GBM pathogenicity have been studied and described in recent years, much remains to be learned. This is particularly true regarding the function of microRNAs (miRNAs) which are aberrantly expressed in GBM (but also in every human cancer) [3,4]. miRNAs open an exciting and promising area for the development of new therapeutic targets as they represent strong regulators of a wide variety of tumorigenic processes including cellular proliferation, differentiation, angiogenesis, invasion, and apoptosis [5,6].

miRNAs are small non-coding, single-stranded RNAs of ~22 nucleotides that modulate the expression of multiple target genes at the posttranscriptional level by binding with target mRNA sequences in the 3'untranslated region (UTR) [5]. miRNAs inhibit multiple target mRNAs by weakly binding to complementary sequences in their 3'-UTRs or through miRNA-directed mRNA cleavage. They interact with numerous mRNA transcripts and, consequently, represent a key molecular checkpoint in the control of crucial biological processes. Profiling studies have shown that miRNAs are differentially expressed in brain tumors compared to normal tissues and such deregulated miRNA subsets could have diagnostic implications [7]. Remarkably, classification of multiple cancers on the basis of miRNA expression pattern was found to be more accurate than using expression patterns of mRNAs [7-13]. Moreover, some reports have already suggested that targeting miRNAs could be more efficient that targeting cell signaling pathways [7,8]. A proof-of-concept for systemic delivery of tumor-suppressing miRNAs as a powerful and highly specific anti-cancer therapeutic modality has been already provided [14-16]. The capacity of GBM cells to infiltrate the healthy surrounding tissue and its subsequent colonization of the entire brain is one of the most life-threatening aspects of the disease. Indeed, when it is possible, surgery allow the partial or total excision of the primary tumor site but not the disseminated cancer cells and the distant secondary tumor site. The lack of relevant models is one of the major limitations in the understanding of the profound and key mechanisms that govern GBM cell fate and biology when they interact with the healthy parenchyma. Indeed, numerous studies used neurospheres as the solely and unique human 3-D system to identify interesting and critical miRNAs [16]. Mostly, these studies compared cancer and normal neurospheres then indentified the most differentially expressed miRNAs. Unfortunately, none of them ever placed the cancer stem cells in their real context: the human brain tissue. It has been widely described that cancer cells behave differently in 2-D compare with 3-D. We have recently showed that even in 3-D cancer cells behave differently when they develop in the host tissue. Indeed, we developed an *in vitro* model of human GBM development within a human brain-like tissue [17]. This system model is based on a combination of patient derived-glioma stem cells and brain-like tissue engineered from human embryonic stem cells (engineered neural tissue, ENT) [17-19]. Our results describe a tumor cell mass growing *in vitro* that exhibits a high degree of similarity to the *in vivo* brain tumor development in GBM patients. This system is fully human (human cancer cells in a human neural tissue) and mimics features of the *in vivo* tumor/host tissue interaction, notably diffuse invasion of the host tissue by tumor cells, formation of secondary tumors and presence of cell death areas [17-19]. To the best of our knowledge this model is unique and represents the only opportunity to study the early events occurring when the GBM cells invade the host tissue in a complete human context. We therefore took advantage of this system and GBM specimens to analyze the entire miRNome through ultra-deep sequencing [19]. A short list of the most differentially expressed miRNAs came out from this integrated analysis and we focused on miR340. Indeed, miR-340 has been found down-regulated in tumors interacting with their host tissue, which is correlated with a better survival [19]. Biologically, miR-340 decreases GBM cell proliferation, adhesion to extracellular matrix and invasion of the host nervous tissue. Moreover, it regulates miR-1293 and

miR-494 (also identified as critical miRNAs in this study) which also inhibit tumor cell proliferation, clonogenicity and invasion [19]. Importantly, the transcriptome analysis showed that GBM cells transfected with the miR-340 mimics expressed a completely different set of genes when they are in 2-D compared to the same transfected cells when they are growing in the ENTs. These results confirmed that GBM cell regulation by miRNAs is highly influenced by the environment and/or the spatial organization of cells in tissues. Indeed, miRNAs expression regulates different set of genes in 2-D and in 3D [19]. MiR-340 has been described and studied by several groups [20-22]. Huang et al. showed a decrease of miR-340 expression in GBM tissues compare with normal brain [20]. Kaplan-Meier survival analysis revealed that low expression of miR-340 were correlated with short overall survival and progression free survival implicating miR-340 as a potent prognosis marker for GBM. Transfection of miR-340 mimics induces a decrease of T98G and A172 cell viability as well as colony formation. Mechanistically, the authors showed that miR-340 over-expression induces a cell cycle arrest at G1/S phase. In this study, EZH2 and CCND1 have been involved as regulators of cell cycle whereas pAKt and EGFR have been implicated as oncogenes playing a function in glioma growth [20]. Moreover, the overexpression of miR-340 induced a decreased of cell motility and invasion toward the modulation of VEGF and MMPs. However, they authors did not comprehensively analyzed the gene expression profile but arbitrary tested few molecules related with motility, growth, cell-cycle in glioma disease. Yamashita and colleagues showed that miR-340 expression was decreased in human and mouse glioma cancer stem cells (CSC) called Glioma Initiating Cells (GICs) [21]. The same correlation between cell motility and invasion with miR-340 overexpression has been showed in this study. They also observed that glioma cells overexpressing miR-340 became senescent as their morphology changed and they confirmed an accumulation of cells in G1 phase and an increase of SA-β-gal staining compare with the control. Histological analysis of miR-340 overexpressing hGICs three days post transplantation showed positivity for caspase-3 immunostaining suggesting that miR-340 induces apoptosis. Transcriptome analysis identified two signaling pathways regulated by miR-340: p21Cip1/CyclinA and cell adhesion/ECM remodeling (PLAT and MMPs). PLAT has been choosen as the principal target because its expression was decreased in miR-340 overexpressing hGICs and its mRNA contained potential miR-340 target sequences (Figure 1). Finally, the authors showed that PLAT promotes hGICs aggressiveness by mediating the function of miR-340 [21]. Li et al. also showed that miR-340 ectopic expression induces a decrease of glioblastoma cell proliferation. They identified CDK6, Cyclin D1 and D2 as targets of miR-340. The expression of one them can rescue glioblastoma cell proliferation and cell cycle arrest mediated by miR-340 down-regulation. In our study, we found that miR-340 modulates genes involved in cell metabolism, cell adhesion/invasion as well as genes involved in cell cycle and proliferation in 2-D. In another hand, once placed on the neural tissue, miR-340 regulates genes involved in host-immune response and genes belong to the family of metallothionein. Accordingly, our results revealed differentially expressed genes in GBM cells (2-D) compare with GBM developing in ENT (3-D).

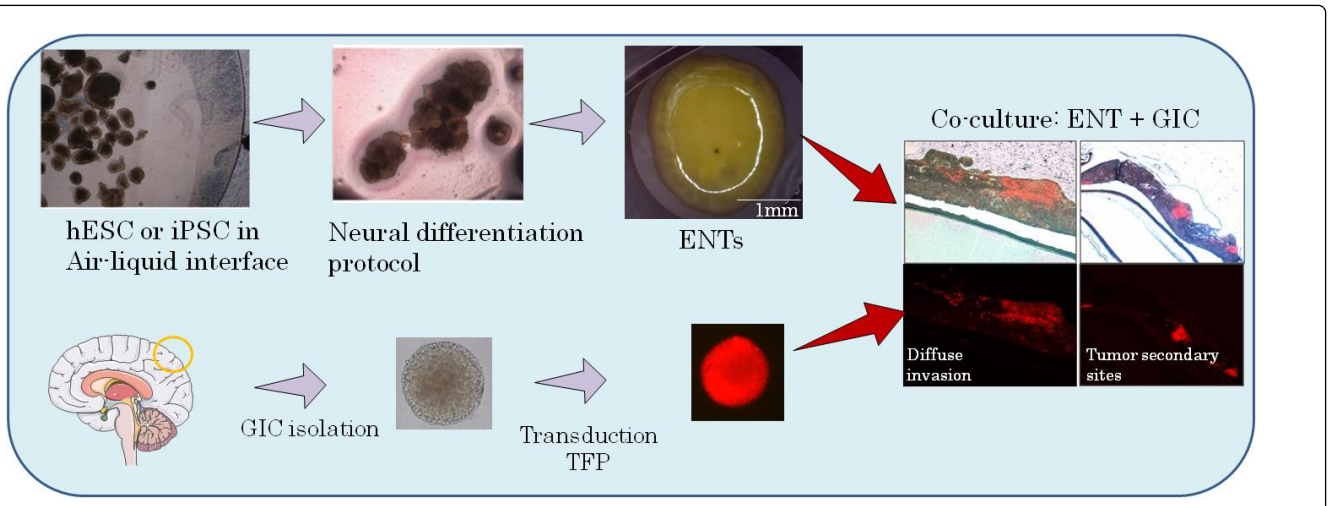

Figure 1: hESC (or iPSC) colonies were detached with accutase and cultured in suspension in ultra-low attachment plates for 4-6 days in neural induction medium. After four weeks, about 30 hESC-derived clusters were plated on a hydrophilic polytetrafluoroethylene (PTFE) membrane deposited on a Millicell-CM (0.4mm) culture plate insert designed for 6-well plates. One milliliter of neural induction medium was added to each well under the membrane insert for differentiation during 4-6 weeks. Glioma-initiating cells (GICs) can be transduced for imaging (Tomato Fluorescent Protein, TFP) and apply on the top of the tissue for 2 weeks of co-culture.

Taken together, we showed that human engineered tissues are not only an opportunity to get insight in the transcriptome and miRNome regulation when GBM cells are in interaction with them but also relevant and elegant model to study tissue interaction dynamics.

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