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It is well established that many solid cancers have complex histology reminiscent of tissues. \(^1\) Cellular phenotypic heterogeneity within tumors may be explained by a hierarchy of differentiation comparable to normal tissues, with only subsets of stem cell-like cells being capable of long-term self-renewal. \(^2\) This idea was first proposed in the 1960s in seminal studies from Pierce and co-workers, who proposed that human tumors may be caricatures of normal embryogenesis. This raises the prospect that signals promoting cell differentiation (i.e., the acquisition of specialized cellular functions) would be effective at driving malignant cells to a less aggressive, and ideally postmitotic, state. Thus, differentiation therapy for human cancers has long had a seductive appeal given the possibilities for limiting tumor growth without the pervasive damage to healthy tissues that is a feature of many chemotherapies.

Glioblastoma multiforme (GBM) is an aggressive primary brain cancer that is driven by cells with neural stem cell characteristics. Bone morphogenetic protein (BMP) signaling is known to trigger cell cycle exit and astrocyte differentiation in GBM stem cells \(^3\) and might therefore be useful as a differentiation therapy. However, it has remained unclear whether BMP can drive GBM stem cells to undergo terminal differentiation and permanent cell cycle arrest. Also, the nature, dynamics, and stability of epigenetic changes that accompany GBM stem cell differentiation have not previously been defined. In a recent study we compared genome-wide changes in transcriptional, DNA methylation, and chromatin accessibility patterns in differentiating GBM-derived neural stem (GNS) cells and genetically normal neural stem (NS) cell controls to assess the stability of the differentiated state. \(^4\) Such studies are important to understand the potential barriers to implementation of successful differentiation therapy for GBM.

We explored BMP4-induced cell cycle exit and concomitant upregulation of astrocyte differentiation markers (glial fibrillary acidic protein [GFAP] and aquaporin 4 [AQP4]), across a large panel of different primary patient GBM stem cell lines. It was immediately apparent that many cultures fail to fully respond to the cytostatic influence of BMP. Many patients will therefore possess tumor stem cells that fail to respond at all to BMP. Perhaps more importantly, even in those cultures where cells were driven out of cycle, we found that DNA methylation was incomplete and/or occurred with delayed kinetics compared to normal NS cells. Likely as a result of this, these non-cycling and overtly differentiated astrocytes or oligodendrocyte-like cells could readily re-enter the cell cycle when challenged with growth factors, and rapid downregulation of differentiation markers ensued. This suggests that cells fail to achieve differentiation commitment and remain vulnerable to de-differentiation (Fig. 1). Thus, although GBM stem cells can engage in programs of differentiation, BMP signaling and/or growth factor withdrawal are not sufficient to drive terminal cell cycle arrest.

Chromatin accessibility mapping (ATAC-Seq) revealed that loci that failed to change in response to BMP were enriched in Sry-related high-mobility-group box (SOX) transcription factor-binding motifs. Thus, increased activity or levels of SOX transcription factors may therefore be part of the restriction on differentiation commitment. It seems that while GBM stem cells can undergo overt morphologic and phenotypic changes indicative of differentiation, they remain immature or partially differentiated. This is clearly highly undesirable, as tumor cells need to be eradicated or permanently driven out of cycle to avoid the risks of further epigenetic and genetic selection for the proliferative stem cell state.
A caveat of our studies is that our assays were based on cell cultures. We also challenged cells to de-differentiate through delivery of a supraphysiological level of growth factors, and it is possible that in vivo tumor cells would not encounter such signals and could remain dormant. However, a latent capacity for dedifferentiation simply by exposure to growth factors has to be concerning for any proposed differentiation therapy. Furthermore, astrocytes and oligodendrocytes have a long lifespan, and therefore any differentiation therapy for GBM needs to account for the fact that the cells will remain long term. Recently, we have also noted that a differentiation-resistant clone characterized by neurofibromin 1 (NF1) deletion was enriched in one of the cell lines during BMP-induced differentiation (unpublished observation). NF1 is an important negative regulator of the Ras signal transduction pathway and NF1 gene mutations and deletions are frequent in GBM, especially within the mesenchymal subgroup. Genetic selection of differentiation-resistant subclones is therefore yet another hurdle that should be anticipated in any clinical design.

We noted unexpectedly slow kinetics of changes in DNA methylation once cells had engaged in astrocyte differentiation for both normal NS cells and subsets of responsive GNS cells. This contrasts with the rapid accumulation of changes that occur in hematopoietic stem cells. One could speculate that the urgent and demanding requirements and turnover within the hematopoietic system necessitate rapid and synchronous decision making, whereas the time course of development and acquisition of critical specialized function in the nervous system occurs over a much longer time frame. Suva et al. recently described a set of specific transcription factors capable of reprogramming non-tumorigenic serum differentiated GBM cells (proneural subgroup) into stem-like tumor-propagating cells. However, the nature of the specific inductive signals in serum are that limited to tumor initiation in contrast to BMP-induced differentiation and whether these findings are limited to subsets of GBM stem cell cultures remain unclear.

To summarize, while it is clear that GBM stem cells can undergo differentiation, it is less clear whether they are able to undergo stable differentiation commitment and terminal cell cycle arrest. Clinical translation of BMP-based differentiation therapies may therefore be difficult. First, there are clear differential responses between cell lines and the same is expected in patients. Second, induction is rapid but the methylation changes are slow and require long exposure. Third, the acquired alterations are not permanent and there is the possibility of reversion, de-differentiation, or selection of differentiation-resistant clones. Clearly, tackling these obstacles up front will be a major challenge in the design of differentiation therapies. Improved understanding of the mechanisms by which tumor cells evade differentiation commitment is necessary for implementation of differentiation therapy for GBM.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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