

REVIEWER 1

You have done a nice job reviewing important concepts. My principal concern is that you presuppose a firm understanding of cancer biology that may elude most of the readership. I would like to see you explain the basic biologic underpinnings more clearly. For example, for concept 7, many readers may not be familiar with DSBs, PARP inhibitors, homologous recombination, etc.

Response: We appreciate this comment and have modified the text of Concept 7 accordingly. In the revised manuscript, we not only explain the three terms highlighted by the referee (DSB, PARP inhibitors, homologous recombination), but also additional ones that might be less familiar to the readers. At the same time, such explanations cannot be too detailed, given the restrictions on valuable journal space (referee no. 3 recommended an overall shortening of the manuscript), and the fact that references are indicated that can help readers to find more information, if desired. We are also pleased to note that reviewer 3 particularly liked the description of Concept 7.

Some minor comments:

Page 4, line 56 - this sentence does not make sense.

Response: We thank the reviewer for this comment. The sentence “While this adage is based on clinical and pathologic observations, systemic genomic characterization of a large number of glioblastoma specimens (The Cancer Genome Atlas project: TCGA) confirms the notion that subtypes with distinct pathologic molecular events and therapeutic response” has been modified to “While this adage is based on clinical and pathologic observations, systemic genomic characterization of a large number of glioblastoma specimens (The Cancer Genome Atlas project: TCGA) confirms the notion that subtypes with distinct pathologic molecular events and therapeutic response exist.” This change can now be found on page 4 of text.

Page 6, line 3 (and accompanying figure) - I don't think the Verhaak paper really demonstrates that patients with the proneural subtype don't benefit from RT/TMZ. All it shows is that they don't benefit from prolonged post-RT chemotherapy.

Response: We thank the reviewer for raising this important point. The Verhaak dataset segregated the patients with various molecular subtypes into two groups: 1) those that received concurrent chemo-radiation therapy or received >3 cycles of chemotherapy and 2) those that did not receive concurrent chemo-radiation therapy or received <4 cycles of chemotherapy. When stratified this way, the authors found that the two groups exhibited comparable survival in the pro-neural group. In contrast, for other molecular subtypes, patients in group 1 exhibited improved survival relative to group 2. (Figure 5, Reference 13). Since the survival effect of concurrent chemo-radiation therapy and prolonged chemotherapy was grouped in the Verhaak analysis, it is difficult to assess whether the effect was due to the former or the latter. In this context, we have modified the text in our review to reflect the data rather than attribute the survival effect to either concurrent chemo-radiation or prolonged chemotherapy. This change can now be found on page 16 of text.

Page 15, line 50: this is inaccurate. First, these are phase II clinical trials of bev, not case series. Secondly, the studies were not designed to assess overall survival. Finally, the improved PFS was really compared to historical controls with ineffective chemotherapy (which included TMZ, which never got approval for recurrent GBM).

Response: We thank the reviewer for raising this point. The thesis that we wished to present is that bevacizumab has not been subjected to the rigor of randomized control trial. We have corrected the information to emphasize that the bevacizumab clinical data were grounded on phase II clinical trials, comparing progression free survival to historical controls of patients who received temozolomide at recurrence. This change can now be found on page 16 of text.

REVIEWER 2

The manuscript is a review of glioblastoma pathobiology. It is well written and contains the key elements. As a review, it is not particularly novel, but provides a compelling point of view.

Response: We appreciate the reviewer's kind remarks.

REVIEWER 3

The authors aim at providing a scholar review on concepts and hallmarks of carcinogenesis, and potential strategies for treatment of glioblastoma. Although the topic and outline of the paper is timely and attractive, they fail to a large extent to meet their goals and respond to the promise of the title. The text is to a large extent lengthy and in parts “boring”, inhomogeneous (e.g. concept 7 is a favorable exception), often sensational using buzz words without providing the scientific insight. The illustrations are not really helpful, while an original art depicting their “concepts and targets” is missing. The next would greatly gain by shortening and English language revision. Repeatedly, sentence are incomplete or meaningless.

Response: We apologize for the lengthy discussions and the meaningless sentences. We have gone through the text to shorten the text where appropriate and refine the language of the manuscript.

Specific Comments:

I would propose to change the title to New concepts in glioblastoma therapy. I don't see why stating the 7 is informative or important. (It is like if Hanahan et al would be saying that there are x hallmarks of cancer.)

Response: In accordance to the reviewer's recommendation, we have modified the title of review to “key concepts in glioblastoma therapy”

There are cases where the phrases lack any meaning (for example: page 4 line 56-60: ... , systemic genomic characterization of a large number of glioblastoma specimens confirms the notion that subtypes with distinct pathologic molecular events and therapeutic response. This is meaningless. There are also repetitions of sections (Page 4 line 8-13 Despite some progress...) It has already been stated.

Response: We thank the reviewer for these comments. The sentence “While this adage is based on clinical and pathologic observations, systemic genomic characterization of a large number of glioblastoma specimens (The Cancer Genome Atlas project: TCGA) confirms the notion that subtypes with distinct pathologic molecular events and therapeutic response“ has been modified to “While this adage is based on clinical and pathologic observations, systemic genomic characterization of a large number of glioblastoma specimens (The Cancer Genome Atlas project: TCGA) confirms the notion that subtypes with distinct pathologic molecular events and therapeutic response exist.” This change can now be found on page 4 of text.

In accordance to the reviewer’s comment, the redundant information on page 4 lines 8-13 has been deleted to achieve a shortened manuscript.

Page 3 line 58 genes when inactivated or activated and contribute to carcinogenesis are always and not generally called oncogenes and tumor suppressors.

Response: We have incorporated the reviewer’s comment into our text.

**Concept 1 Phrase without sense: This profiling approach...
Consider adding to concept 1: that while subtypes are predictive they not seem to be prognostic.**

Response: We have revised the phrase in question to the following “These studies have led to the understanding of glioblastoma as an umbrella term that encapsulates subtypes characterized by distinct molecular properties.”

The aggregate of the data suggests that the transcriptome based molecular subtypes are both predictive and prognostic. Philips et. al. (Reference 12) as well as Verhaak et. al. (Reference 13) both yielded evidence that patients with the pro-neural subtype of glioblastoma survive longer than those with other molecular subtypes. Verhaak et. al. (Reference 13) further demonstrated that the patients with pro-neural subtype of glioblastoma tend not to benefit from concurrent chemo-radiation therapy or prolonged chemotherapy. We have further clarified this on page 5.

Concept 2. When is a cell hyper-dependent and when only dependent? Please avoid hyper-dependence. Hyper-activation though does exist.

Response: We thank the reviewer for this critical question. We define “hyper-dependence” as a term to describe a situation where the tumor cell is more dependent on a particular process than the non-neoplastic cell. The definition can now be found on page 9 of the manuscript.

Please provide the proper references to genetic streamlining etc. and not only the review of Sharma et al from Genes Dev. 2007 Dec 15;21(24):3214-31. Also if you name the first two theories why not give the name of the third namely, oncogenic shock (Sharma and Settleman 2006)?

Response: We have cited two other 2006 papers by the Settleman group discussing the hypothesis of “oncogenic shock”. We also included “oncogenic shock” as a terminology in the manuscript per the reviewer’s request (page 8).

Page 6 line 51. Not anti-intuitive but contra-intuitive

Response: According to Merriam-Webster’s Collegiate Dictionary, the proper term is neither anti-intuitive nor contra-intuitive. The correct term of “counter-intuitive” has been changed on page 7.

Concept 3. No comment, correctly written

Response: We thank the reviewer for the kind review

Concept 4. There is clear evidence also against TICs which should be stated including Quintana et al Nature. 2008 Dec 4;456(7222):593-8. Also Indar Verma’s group has shown that it is also true for glioblastoma. It is exactly the current test (glioblastoma formation in xenografts) which is limiting the understanding of the true nature of TICs.

Response: We thank the reviewer for this comment. We whole-heartedly agree that the current technology is limited for the study of TICs. However, with all due respect, we disagree with the reviewer on the interpretation of the Quintana paper as clear evidence against the existence of TIC. The paper reports that, on average, 27% of single cell suspensions derived from melanoma patients are capable of forming xenograft tumors when implanted into severely immunocompromised mice. The data may suggest that the prevalence of TICs in melanoma cells may be higher than previously thought when severely immunocompromised mice are used as an assay for assessing TIC activity. However, this data set does not constitute evidence against the existence of TICs. This discussion has been appended on page 10.

To our best knowledge, Dr. Verma’s result has not been published in a peer-reviewed journal. In this context, we cannot include this information in the current review.

Concept 5. In concept 5 the authors mix up tumor heterogeneity with microenvironment. Both are important but in the current presentation it is misleading. It is not clear from the text which cells express IL6 and LIF and instead of transactivation I would suggest to use paracrine activation as it is noted correctly later. The authors explain endothelial cells but there is no sign of the VEGF signaling and when actually they present VEGF there is no explanation what it could be.

Response: We thank the reviewer for this comment. We had intentionally conceptualized heterogeneity in tumor cell as part of the tumor microenvironment. This thesis has been better stated on page 12.

We apologize for not clearly stating the source of IL6 and LIF. These factors are secreted by EGFRvIII cells. This information has been appended on page 13.

The discussion of VEGF signaling was moved up to page 14 in accordance to the reviewer's comment.

PTEN modulates Akt phosphorylation and not the S6Kinase. The effect o S6K kinase is indirect as it is lower on the pathway. Many other signaling regulates S6 kinase. As written PTEN is regulating the immune inhibitory cytokines but it is not clear which way therefore it is not at all necessary (as written on page 15 that PTEN loss will increase IL10 and B7-H1.

Response: We thank the reviewer for the suggestion of revision and have revised accordingly on page 15.

The authors suggest that there were case series comparing temozolomide with bevacizumab, but none of the provided references appear correct. Indeed the references presented are about bevacizumab +/- irinotecan in recurrent glioblastoma, after failure of temozolomide. Therefore the two cannot be compared.

Response: We thank the reviewer for bringing up this important point. The information has been corrected to "While there has not been a randomized control trial to assess the efficacy of bevacizumab, phase II clinical trial demonstrated improved progression free survival in recurrent glioblastomas (after concurrent temozolomide/radiation treatment) relative to historical data reported based on patients who received temozolomide at recurrence". This can be found on page 16 of the revised manuscript.

Concept 6. In concept 6 authors mix up again two distinct phenomena, non-coding RNA with epigenetic modifications. These should be discussed as separate entities. From the later the authors arbitrary choose miRNAs and lincRNAs. It should be specified that this are a part of 2 bigger groups of non-coding RNA. LincRNAs (large intergenic non-coding RNA which should be noted in the text) are a part of the family of LncRNAs, long non-coding RNAs, whereas miRNAs is a part of the short regulatory RNAs including, siRNAs, piRNAs and snoRNAs. All this are potentially important in carcinogenesis.

Response: We appreciate this comment. For our review, we adopted the classical definition of coding sequence as the strand of DNA that has the same base sequence as the RNA transcript produced (with the caveat that thymines are replaced by uracil). To the extent that promoter regions are not part of this coding sequence, we discussed promoter methylation under the general heading of non-coding sequences. This discussion has been appended on page 16 of the revised manuscript.

We recognize the distinction between LincRNAs and miRNAs. To the extent that these sequences are non-coding by the classic definition, we discussed these entities in the section of non-coding RNAs.

Page 17 linne 3 There are no different patterns of promoter methylation. MGMT methylation is a single marker of TMZ responsiveness and it is not a pattern, while G-CIMP is a phenotype presented by a subgroup of patients.

Response: We appreciate the reviewer's comment that MGMT promoter methylation and G-CIMP phenotype are distinct biomarkers for glioblastoma patients. We had conceptualized these events as distinct patterns of CpG island methylation. To accommodate the reviewer's perspective, we have modified the sentence to "There are two types of promoter methylation that are particularly pertinent to glioblastoma therapy" on page 17 of the revised text.

Page 17 line 38. The sentence is non-sense and does not explain the better prognosis of MGMT methylated patients.

Response: We apologize for not explaining this concept more clearly. As explained in concept 7 and seen in reference 30, glioblastoma cells accumulate endogenous DNA damage in the absence of DNA damaging agents. These endogenous DNA damages are not unlike those induce by temozolomide or radiation in that they could trigger cell death if unrepaired. Thus, tumors with high levels of MGMT may grow more robustly since MGMT is capable of detoxifying many of these endogenous DNA damages. If the tumor cells grow more robustly, the patient will survive for a shorter duration. In contrast, the glioblastoma cells with low MGMT may be more susceptible to the deleterious effects of the endogenous DNA damages. These tumors may grow less robustly, resulting in longer patient survival. This explanation has been incorporated into page 18 of the revised text.

Page 18 How would you selectively enhance promoter methylation? Please explain. LincRNA,,: please specify what it stands for. Change: These RNAs do not encode for proteins... to these RNAs are transcribed by Pol II but do not encode proteins. If LincRNA is not yet implicated in GMB why discuss?

Response: We thank the reviewer for this question. Recent studies suggest that promoter methylation at distinct loci may be affected by specific chromatin modulating factors. We have included this into the discussion on page 18.

LincRNA has been defined as Long Non-Coding RNA.

The suggested change (These RNAs do not encode for proteins... to these RNAs are transcribed by Pol II but do not encode proteins.) has been made on page 19 of the revised text.

To the extent that LincRNA play important roles in mediating p53 functions, and p53 plays a pivotal role in the pathogenesis of glioblastomas, we feel that a discussion of LincRNA is warranted. This discussion is added to page 19 of the revised text.

Concept 7 well written, no comment

Response: We thank the reviewer for the kind review