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Peer Review

Review of: "Kinase Suppressor of Ras 2 Promotes Self-Renewal and Clonogenicity of Small-Cell Lung Carcinoma"

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Review of Manuscript Entitled: Kinase Suppressor of Ras 2 Promotes Self-Renewal and Clonogenicity of Small-Cell Lung Carcinoma

Summary of Findings:

The manuscript presents interesting findings on the role of Kinase Suppressor of Ras 2 (KSR2) in the self-renewal and clonogenicity of small-cell lung carcinoma (SCLC). While the study provides valuable mechanistic insights, several aspects of data presentation and experimental clarity could be improved to enhance the manuscript's readability and scientific rigor.

Major Comments:

1. **Figure Presentation and Labeling:**
    - **Figure 1A-C:** The y-axis scaling is unclear and should be adjusted for better interpretation. Even though the graphs all represent gene expression levels, sometimes the y-axes have normalized log2 counts, sometimes FPKM, and sometimes expression relative to GAPDH. If possible, keep the axis scales consistent.
    - **Figure 1H:** The data clearly demonstrate higher expression in ASCL1 tumors and heterogeneous expression in other subtypes. Adding a label to indicate that the x-axis represents lung tumor subtypes would improve clarity for people unfamiliar with the lung cancer field. The same applies to Figure 1F (add that those are mouse-model derived cell lines).

doi.org/10.32388/DDC8UT

- **Figure 2:** The role of KSR2 in colony formation is well demonstrated, but figure labels need clarification. Specifically:
  - **Figure 2A:** What is the take-home message from the FACS plots? Would move to SI.
  - **Figure 2E:** What constitutes the control condition? Is it shScramble+DOX or sh6/7-DOX?
  - **Figure 2F:** Make sure that the control (sh5-DOX) is consistent with what was used in Fig. 2E compared to sh5+DOX, and how do these conditions align with other experiments?
- **Figure 3:** This is a very well-designed and interesting experiment. The mechanistic experimental design is robust and contributes to understanding KSR2 function.
- **Figure 5:** It would strengthen the manuscript to determine whether the findings hold true in patient-derived xenografts (PDXs) from human cell lines.
- **Figure 6:**
  - **Panels A-C:** The differences in pERK levels would be clearer by showing a quantification.
  - **Panel B:** The blot appears overexposed; a clearer representation is needed.
  - **DOX Treatment:** Specify what DOX treatment activates (e.g., shRNA, sgRNA) and which constructs are affected.

2. **Experimental Consistency:**
   - The choice of cell lines is inconsistent, with some experiments using shRNAs and others using sgRNAs. Not all conditions are tested consistently across mouse-model-derived cell lines and human lines. Standardizing or justifying these choices would improve interpretability.
3. **Mechanistic Claims and Contradictions:**
   - One of the manuscript's major claims is that KSR2 enhances tumor initiation through its interaction with ERK but not via ERK activity. However, ERK activity is assessed only using trametinib. Additional evidence is needed to support this claim, such as testing other MAPK pathway inhibitors.
   - Contradictory findings need clarification:
     - KSR2 appears to reduce clonogenic capacity by disrupting ERK activation (which is supported by the finding that tumor-propagating cells demonstrate slight but higher ERK activity).
     - However, ERK inhibition via trametinib does not affect TPC frequency, raising questions about the proposed mechanism.
     - RNA sequencing (RNA-seq) following KSR2 knockdown and in the presence of the ERK-binding mutant would provide valuable insights into the pathways that are upregulated or

downregulated.

4. **Additional Considerations:**
    - The use of schematics for experimental workflows would improve readability, as the text currently contains excessive abbreviations for people unfamiliar with the lung cancer field.
    - The discussion could be more concise, with a stronger focus on the key findings. Some sections, such as references to cisplatin, do not integrate well with the main study objectives and could be streamlined or omitted.

Conclusion:

The study presents compelling data on KSR2's role in SCLC, but several aspects require refinement. Improved figure labeling, experimental consistency, and additional mechanistic validation will enhance the manuscript's impact. Addressing these concerns will significantly strengthen the clarity and scientific rigor of the work.

Declarations

Potential competing interests: No potential competing interests to declare.