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REVIEWER'S REPORT

Manuscript No.: IJAR- 58484

Title: Computational study on protein interactions of CDKN2C and its impact on cancer studies

Recommendation:
Accept after minor revision

Rating	Excel.	Good	Fair	Poor
Originality		✔		
Techn. Quality			✔	
Clarity			✔	
Significance	✔			

Reviewer's ID: Bilqees Hamza

Detailed Reviewer's Report

The manuscript investigates the interactions, co-expression, and evolutionary conservation of the *CDKN2C* gene/protein (p18^INK4c^) and its potential implications for cancer biology, specifically regarding osteosarcoma, multiple myeloma, and small cell lung carcinoma (SCLC). The author utilizes computational tools—specifically the STRING database for protein-protein interaction (PPI) networks, k-means clustering, gene co-occurrence, and co-expression—alongside the AUGUSTUS tool for gene structure predictions of *RANKL* (*TNFSF11*) and *Matrilin 3* (*MATN3*).

The core topic is relevant to modern computational oncology and biomarker discovery. The integration of network-level protein interactions with literature evidence regarding radiosensitivity and chromosomal deletions presents a worthwhile area of inquiry.

However, the draft in its current state exhibits severe conceptual disjunctions, methodological gaps, superficial analysis, and significant formatting deficiencies. Most notably, large portions of the text and data (such as the *ab initio* gene predictions for *RANKL* and *MATN3*) are completely disconnected from the primary subject matter of *CDKN2C* and cancer pathway analysis. Extensive revisions, narrative integration, and scientific contextualization are required before this paper can meet the standards of scholarly publication.

Key Areas for Improvement

1. Scope, Disconnected Analysis, and Conceptual Coherence

- **Unrelated Sequence Predictions (*RANKL* and *MATN3*):** The manuscript abruptly transitions from analyzing *CDKN2C* interactions to running AUGUSTUS gene predictions on *RANKL* (comparing *Homo sapiens* and *Mus musculus*) and *Matrilin 3* (*MATN3*). There is zero narrative

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explanation or functional biological bridge linking *RANKL* or *MATN3* back to *CDKN2C*, cell cycle regulation, or the identified PPI network. If *RANKL* is included due to its role in osteoclastogenesis and bone microenvironments in osteosarcoma or multiple myeloma, the author must explicitly build this biological hypothesis. Otherwise, these sequences appear as arbitrary inclusions and should be removed.

- **Superficial STRING Network Analysis:** The STRING analysis (Figure 1) applies k-means clustering with $k=6$ and a confidence threshold of 0.400. However, the manuscript merely lists a few interactors (*CDKN1C*, *CDKN2D*, *CCNL2*, *E2F4*) without exploring the biological meaning of the resulting clusters. What are the Functional Enrichment (GO terms, KEGG/Reactome pathways) results for each cluster? What are the hub bottleneck nodes in this network? The analysis needs to move beyond simple visual description to quantitative topological evaluation.

2. Methodological Clarifications and Algorithmic Rigor

- **Clarification of k-Means Parameters:** In Section "k means clustering algorithm and working principle" (lines 41–48), the author provides a textbook definition of k-means clustering. This standard explanation should be condensed. Instead, the author must detail *how* k-means was applied within STRING, what feature matrix (e.g., interaction scores) was used, and why $k=6$ was selected as the optimal cluster number over other values (e.g., via silhouette analysis or elbow method).
- **Gene Co-occurrence and Evolutionary Interpretation:** Figure 2 and lines 38–40 note that *CDK4* and *CDK6* co-occur in Eukaryota but show low occurrence in Archaea. This is an expected biological triviality, as eukaryotic cell cycle machinery is distinct from archaeal cell division mechanisms. The author should focus the co-occurrence and co-expression analysis specifically on higher vertebrates/mammals to draw meaningful conclusions about conserved tumor-suppressor networks.

3. Literature Integration and Discussion Depth

- **Inadequate Synthesis:** The Introduction reviews literature on *CDKN2C* in radiosensitivity, multiple myeloma 1p32.3 deletions, and SCLC prognosis. However, the manuscript completely lacks a dedicated "Discussion" section. The results from STRING are not critically evaluated against these published clinical observations.
- **Biomarker Hypothesis:** The Conclusion states that *CDK4* and *CCND1* can serve as biomarkers for targeting cell cycle progression in *CDKN2C*-deficient cells. The author must elaborate on the mechanistic rationale: How does loss of $p18^{\text{INK4c}}$ (encoded by *CDKN2C*) lead to

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synthetic vulnerability or altered sensitivity to CDK4/6 inhibitors (such as Palbociclib or Ribociclib) in combination with Cyclin D1 expression?

4. Formatting, Typography, and Figure Quality

- **Mathematical and Notation Formatting:** Standard scientific conventions must be maintained throughout the text:
  - Gene names (*CDKN2C, CDK4, CCND1, RB1*) should be consistently italicized, whereas protein names (p18, CDK4, Cyclin D1) should remain in standard Roman type.
  - Numerical percentages are missing correct punctuation (e.g., line 20 states "7 to 4%" instead of "7% to 4%" or "74%" depending on the cited source).
  - Chromosomal locations must follow standard notation (e.g., 1p32.3).
- **Raw Sequence Dumps:** Lines 65–132 consist of raw FASTA nucleotide and amino acid sequence dumps. In a journal article, unformatted raw sequence blocks are inappropriate. These should be summarized in a comparative alignment table (e.g., BLAST alignment percentage identity, domain conservation) or moved to Supplementary Information.
- **Figure Resolution and Labels:** The provided figures (Figure 1, Figure 2, Figure 3) suffer from low resolution, pixelation, and illegible node labels. High-resolution vector or high-DPI raster images must be generated directly from STRING, with clearly readable gene symbols and legend keys.

Recommendation

Minor Revision Required.

The underlying computational approach holds potential for highlighting the functional interaction network of *CDKN2C* in bone and epithelial malignancies. However, the current draft is fragmented and incomplete. The author must address the following points in a revised submission:

1. **Remove or Integrate Non-Sequitur Sections:** Either establish a clear, mechanistically justified link between *CDKN2C* and *RANKL*/*MATN3* in bone malignancies, or remove the AUGUSTUS sequence predictions entirely to focus strictly on the cell-cycle interaction network.
2. **Perform Advanced Network & Pathway Enrichment:** Expand the STRING analysis to include Functional Enrichment (GO Terms for Biological Processes, KEGG Pathways) for the k-means clusters. Identify key hub genes and bottleneck nodes.
3. **Add a Dedicated Discussion Section:** Synthesize the computational findings (*CDK4, CCND1, CDKN1C, E2F4*) with clinical evidence on radiosensitivity and 1p32.3 deletions in osteosarcoma and multiple myeloma.

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4. **Re-format Sequences and Figures:** Replace raw sequence blocks with comparative alignment summaries and provide crisp, high-resolution figures with fully legible text.
5. **Thorough Editorial Polish:** Correct all typographical errors, standardize gene/protein nomenclature, and refine the prose for academic clarity.

Final Evaluation

Recommendation: Accept with Minor Revisions