



SDI Review Form 1.6

Journal Name:	International Research Journal of Oncology
Manuscript Number:	Ms_IRJO_43634
Title of the Manuscript:	Differential miRNA expression in oral cancer oncosomes: a pilot study.
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	<p>Introduction: the objective of the paper should be at <u>the end of Introduction</u>, clearly stated and expressed in an orderly fashion.</p> <p>M&M: were the particular SCC lines for the study selected for a reason? (availability, representativity, or something else?). This should be clarified.</p> <p>- “<i>Thermo Scientific Verso 1-Step RT-PCR</i>” [5th paragraph] This line is not connected, please clarify. What was the PCR kit used? Were more than one?</p> <p>- “<i>the relative-fold expression</i>” the process for obtaining this should be detailed clearly (e.g. Normalization), as to why the references low, moderate and High. Why there are not any statistical analysis performed?</p>	<p>1. The authors have reviewed this comment and concur – the Introduction has been revised and the final sentence now reads:</p> <p>To date, only one study had demonstrated the secretion of oncosomes from cultured OSCC cells, therefore the objective of this study is to determine if intact oncosomes can be isolated from oral cancer cells <i>in vitro</i>.</p> <p>2. The authors concur and have revised the Methods to reflect the use of already purchased (and therefore available) cell lines. The revised text now reads:</p> <p>Cell lines that were available for this study (already purchased and cultured) included the human oral squamous cell carcinoma lines, SCC-4 (CRL-1624), SCC-9 (CRL-1629), SCC-15 (CRL-1623), SCC-25 (CRL-1628), and CAL-27 (CRL-2095) were originally obtained from the American Type Culture Collection (ATCC: Manassas, VA). The normal human gingival fibroblast cell line HGF-1 (CRL-2014) was also previously obtained from ATCC and used as a normal control for comparison.</p> <p>3. This kit was used and this particular sentence was meant to have a colon instead of a period. The revised text now reads:</p> <p>Thermo Scientific Verso 1-Step RT-PCR: Reverse transcription and PCR were carried out together in a single 25 µL reaction containing 2.5 µL of exosomal RNA, the desired forward and reverse primers, RT enhancer, 2X RT-PCR buffer, and versa reverse transcriptase enzyme.</p> <p>4. Standard curves were made using CAL27 and SCC9 cDNA, which is now described briefly in the methods section, as follows:</p> <p>Standard curves were made doing a five-fold serial dilution of CAL-27 cDNA for miR-365, miR-21 and miR-155 while standard curves for miR-133a was done with SCC-9 cDNA.</p> <p>The categorization into low, moderate and high was comparative and is now described in some detail in the results section, as follows:</p> <p>These quantitative data derived from the qPCR were compiled and microRNA levels categorized with the relative-fold expression (RFE) as Low (RFE < 100), Moderate (RFE =100-1000), and High (RFE > 1000) (Table 2).</p> <p>Because this is an observational study describing relative fold expression, the authors could not justify a statistical analysis of these data. Categorical analysis, such as Chi-square would be inappropriate since there is no “expected” value and correlation would provide no meaningful outcome. In future experiments where an experimental (independent) variable is introduced (drug, inhibitor) and a change in miRNA expression is observed, a robust statistical analysis will be included.</p> <p>The authors are associated with an academic institution and have no business or commercial interests or conflicts regarding this study. The</p>



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		manuscript text now reads:
Minor REVISION comments	Introduction: It would greatly improve the manuscript value if a small part referring to mRNA in oral cancer was added mentioning specific mRNA for it in this section, however brief.	The authors have included a few more of the specific miRNAs that have been found in both oncosomes and exosomes, and the revised text now reads: While over 750 miRNAs such as miR-30, miR-204, miR-370, miR144 and miR-193 have been found in both oncosomes and exosomes, the expression of four specific miRNAs: miR-365, miR-21, miR-155, and miR-133a-1 have been extracted from multiple types of cancers, including oral cancers [5].
Optional/General comments	Interesting study.	