Alteration of MicroRNA Expression Patterns in Cervical Cell Carcinomas towards Chemotherapeutic Agents

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Cervical cancer – one in ten female cancers diagnosed worldwide\(^1\); third most common cancer in Malaysian women\(^2\)

Chemotherapy – dose-limiting toxicity and drug resistance\(^3\). Example: cisplatin (CDDP) used in the treatment of cervical cancer

1’S-1’-acetoxychavicol acetate (ACA) – natural compound extracted from \textit{Alpinia conchigera} Griff. (Zingiberaceae) found to induce apoptosis on various cell lines with no adverse effects on normal cell line\(^4\)

MicroRNAs – small non-coding RNA molecules which regulate many genes post-transcriptionally\(^5\)
Research questions

- What are the effects of ACA and/or CDDP on cervical cell carcinomas?
- Which miRNAs are involved in modulating response towards ACA and/or CDDP?
Materials and Methods

**CYTOTOXICITY ASSAY**

- MTT cell viability assay
  - Stand-alone
  - Combination

**Combination analysis**

- Isobologram
- Combination index

**MIRNA EXPRESSION**

**miRNA microarray**

- FlashTag™ Biotin RNA Labelling Kit
- Affymetrix® GeneChip® miRNA Arrays

**Data analysis**

- Partek® Genomics Suite™ 6.5

**Data validation**

- TaqMan® MicroRNA Reverse-Transcription Kit
- TaqMan® MicroRNA Assays
Both ACA and CDDP induced dose- and time-dependent cytotoxicity on cervical cell carcinoma Ca Ski when used as a stand-alone agent.
Results - Combination Chemotherapy

Tab. 1: Interaction between ACA and CDDP

<table>
<thead>
<tr>
<th>Combination chemotherapy</th>
<th>Drug Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA constant + CDDP variable</td>
<td>Synergistic</td>
</tr>
<tr>
<td>CDDP constant + ACA variable</td>
<td>Synergistic</td>
</tr>
<tr>
<td><strong>ACA constant → CDDP variable</strong></td>
<td>Synergistic</td>
</tr>
<tr>
<td>CDDP constant → ACA variable</td>
<td>Antagonistic</td>
</tr>
</tbody>
</table>

Results indicated that ACA acts as a **chemosensitizer**, with sequential exposure to ACA followed by CDDP being the most optimal combination.
<table>
<thead>
<tr>
<th>miRNA</th>
<th>ACA</th>
<th>CDDP</th>
<th>ACA + CDDP</th>
<th>Function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-let-7g</td>
<td>-2.04*</td>
<td>-1.09</td>
<td>-1.77</td>
<td>Cell cycle arrest and cell death(^6)</td>
</tr>
<tr>
<td>hsa-miR-138</td>
<td>-1.03</td>
<td>+1.21</td>
<td>+2.07*</td>
<td>Multidrug resistance(^7)</td>
</tr>
<tr>
<td>hsa-miR-210</td>
<td>+1.46</td>
<td>+2.37*</td>
<td>+2.18</td>
<td>Cell cycle regulation(^8)</td>
</tr>
<tr>
<td>hsa-miR-224</td>
<td>-2.06*</td>
<td>-1.38</td>
<td>-1.60</td>
<td>Cell death and proliferation(^9)</td>
</tr>
<tr>
<td>hsa-miR-629</td>
<td>+2.16*</td>
<td>+1.27</td>
<td>+1.37</td>
<td>Universal marker in cancer(^10)</td>
</tr>
<tr>
<td>hsa-miR-663</td>
<td>+1.43*</td>
<td>+2.09*</td>
<td>+1.39</td>
<td>Inflammatory response(^11)</td>
</tr>
<tr>
<td>hsa-miR-720</td>
<td>-1.07</td>
<td>+2.18*</td>
<td>-1.20</td>
<td>Marker in renal cell carcinoma(^12)</td>
</tr>
<tr>
<td>hsa-miR-744</td>
<td>+ 1.97*</td>
<td>+1.23</td>
<td>+2.18*</td>
<td>-</td>
</tr>
<tr>
<td>hsa-miR-1244</td>
<td>-1.14</td>
<td>+2.23*</td>
<td>+1.06</td>
<td>-</td>
</tr>
</tbody>
</table>

Tab. 2: List of miRNAs whose expression is altered by administration of ACA and/or CDDP (Note: * p < 0.05)
The miRNAs expression patterns were altered upon administration of ACA and/or CDDP.

Tab. 3: Fold-change in expression of selected miRNAs as compared to untreated controls using qRT-PCR (Note: * p < 0.05)

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<th>CDDP</th>
<th>ACA + CDDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-138</td>
<td>+1.01</td>
<td>+1.44*</td>
<td>+2.17*</td>
</tr>
<tr>
<td>hsa-miR-224</td>
<td>-3.27*</td>
<td>-1.23</td>
<td>-2.43*</td>
</tr>
<tr>
<td>hsa-miR-744</td>
<td>+ 2.48*</td>
<td>+1.69*</td>
<td>+2.27*</td>
</tr>
</tbody>
</table>
Conclusion

- Combination chemotherapy with ACA and CDDP improves cytotoxic effects → ACA acts as chemosensitizer that potentiates the effects of CDDP

- The expression of several miRNAs were altered by the administration of ACA and/or CDDP → suggesting miRNAs are involved in regulating response towards chemotherapeutic agents
Future prospective

- Functional studies – to elucidate how miRNAs regulate response towards chemotherapeutic agents
- This will help to understand the problems associated with chemotherapy – improve the efficacy in chemotherapy
References


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