Alterations of Cancer MicroRNA Expression Patterns in Human Cervical Carcinoma Cells (Ca Ski) towards 1'S-1'-Acetoxychavicol Acetate (ACA) and Cisplatin (CDDP)

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Introduction

- **Cervical cancer**
  - second most common cancer in women worldwide\(^1\)
  - third most common cancer among Malaysian women\(^2\)

- **MicroRNAs (miRNAs)**
  - short non-coding RNA molecules
  - regulate genes negatively at post-transcriptional level
  - implicated in many processes such as cell proliferation and cell death\(^3\)

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**Increasing cervical cancer cases and deaths in GAVI-eligible countries**

![Graph showing increasing cervical cancer cases and deaths in GAVI-eligible countries](source)

Source: Based on GLOBOCAN database www.globocan.iarc.fr and GAVI-eligible countries.
Introduction

- Cisplatin (CDDP)
  - treatment of cervical cancer\(^4\)
  - dose-limiting toxicity and drug resistance\(^5\)

- 1’S-1’-acetoxychavicol acetate (ACA)
  - natural compound extracted from *Alpinia conchigera* Griff. (Zingiberaceace)
  - induces apoptosis on various cell lines without adverse effects on normal cell line\(^6\)

![Chemical structure of CDDP](image1)
![Chemical structure of ACA](image2)

Research Aims

- To investigate the combined effects of ACA and CDDP on Ca Ski human cervical carcinoma cells
- To identify cancer-related miRNAs modulated in response towards ACA and CDDP
- To determine the interactions between miRNAs and target mRNAs
Methodology

Cytotoxicity Assays

MTT cell viability assay
- Stand-alone
- Combination

Combination analysis
- Isobologram
- Combination index

MiRNA Expression

MiRNA microarray
- Affymetrix® GeneChip® miRNA Arrays

Data analysis
- Partek® Genomics Suite™ 6.5

Data validation
- TaqMan® MicroRNA Assays

Pathway Analysis

Target prediction
- TargetScanHuman v5.2

Gene-annotation enrichment
- Database for Annotation, Visualization and Integrated Discovery v6.7
ACA and CDDP induces **dose- and time-dependent cytotoxicity** when used as a standalone agent.
### Combination analyses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ACA (µM)</th>
<th>CDDP (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standalone ACA</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td>Standalone CDDP</td>
<td>-</td>
<td>53.3</td>
</tr>
<tr>
<td>C1</td>
<td>3.0</td>
<td>13.0</td>
</tr>
<tr>
<td>C2</td>
<td>2.5</td>
<td>24.0</td>
</tr>
<tr>
<td>C3</td>
<td>3.0</td>
<td>13.3</td>
</tr>
<tr>
<td>C4</td>
<td>4.8</td>
<td>24.0</td>
</tr>
</tbody>
</table>

**Treatment ACA synergistically potentiates** the cytotoxic effects of CDDP when used in combination.

### Table: Treatment Regimen

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Regimen</th>
<th>Time (h)</th>
<th>Combination index (CI) †</th>
<th>Drug Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Simultaneous: ACA [3 µM] + CDDP [0-100 µM]</td>
<td>24</td>
<td>0.74 ± 0.01</td>
<td>Synergistic</td>
</tr>
<tr>
<td>C2</td>
<td>Simultaneous: CDDP [24 µM] + ACA [0-12 µM]</td>
<td>24</td>
<td>0.87 ± 0.02</td>
<td>Synergistic</td>
</tr>
<tr>
<td>C3</td>
<td>Sequential: ACA [3 µM] → CDDP [0-100 µM]</td>
<td>12 → 24</td>
<td>0.75 ± 0.02</td>
<td>Synergistic</td>
</tr>
<tr>
<td>C4</td>
<td>Sequential: CDDP [24 µM] → ACA [0-12 µM]</td>
<td>12 → 24</td>
<td>1.25 ± 0.05</td>
<td>Antagonistic</td>
</tr>
</tbody>
</table>

*The interaction is synergistic, additive, or antagonistic if the CI is less than, equal to or more than 1, respectively*

†The interaction is synergistic, additive, or antagonistic if the CI is less than, equal to or more than 1, respectively
## MiRNA Expression

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Microarray Fold-change†</th>
<th>p value‡</th>
<th>qRT-PCR Fold-change†</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-138</td>
<td>2.13 ± 1.09</td>
<td>0.049</td>
<td>1.58 ± 0.31</td>
</tr>
<tr>
<td>hsa-miR-744</td>
<td>2.11 ± 1.15</td>
<td>0.045</td>
<td>2.17 ± 0.05</td>
</tr>
<tr>
<td>hsa-miR-210</td>
<td>2.02 ± 0.83</td>
<td>0.033</td>
<td>2.03 ± 0.75</td>
</tr>
<tr>
<td>hsa-miR-523</td>
<td>1.68 ± 0.44</td>
<td>0.044</td>
<td>n/a</td>
</tr>
<tr>
<td>hsa-miR-922</td>
<td>1.67 ± 0.55</td>
<td>0.026</td>
<td>n/a</td>
</tr>
<tr>
<td>hsa-miR-1271</td>
<td>-1.80 ± 0.06</td>
<td>0.002</td>
<td>n/a</td>
</tr>
<tr>
<td>hsa-miR-224</td>
<td>-1.81 ± 0.20</td>
<td>0.048</td>
<td>-1.53 ± 0.06</td>
</tr>
<tr>
<td>hsa-miR-21*</td>
<td>-1.87 ± 0.20</td>
<td>0.046</td>
<td>n/a</td>
</tr>
</tbody>
</table>

†Positive values denote up-regulation while negative values denote down-regulation as compared to untreated controls; ‡ p values ≤ 0.05 were considered significant; n/a denotes data not available.

ACA in combination with CDDP altered expression of 8 miRNAs (5 up-regulated and 3 down-regulated)
Pathway Analysis

miR-138  miR-210  miR-744

Targets of up-regulated miRNAs
Conclusion

• Combination of ACA and CDDP results in synergistic effects → ACA potentiates the effects of CDDP

• ACA and CDDP alters miRNA expressions → miR-138, miR-210, miR-523, miR-744 and miR-922 are up-regulated while miR-21*, miR-224 and miR-1271 are down-regulated

• Candidate miRNAs (miR-138, miR-210, miR-744) target multiple pathways → WNT, ERK, TGF-β, NF-κB, Ca^{2+}, and intrinsic apoptosis

• Interactions between miRNAs and target mRNAs → miRNAs play direct and indirect role in response towards ACA and CDDP → understand molecular mechanisms underlying anti-cancer drugs response

• Potential therapeutic approaches in chemotherapy → exploit miRNA expression to improve efficacy in combination chemotherapy.
Conclusion

**ACA**
- decreases glutathione levels
- inhibits NF-κB activation

**CDDP**
- cell cycle arrest
  - apoptosis

**miRNA**
- down-regulates genes associated with CDDP resistance
- biomarkers to predict response towards chemotherapy

Synergistic effects resulting in:

i) Increased apoptosis

ii) Increased cell cycle arrest

iii) Lower effective dose

iv) Lesser side-effects

v) Lower recurrence of resistance

2) Zainal, AO, Zainuddin, MA, Nor Saleha, IT. (2006) Malaysia Cancer Statistics - Data and Figure, Peninsular Malaysia. National Cancer Registry, Ministry of Health Malaysia.


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