<table>
<thead>
<tr>
<th>Name of the Employee</th>
<th>Period of Employment</th>
<th>Project Investigator (Head of Department/Center)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yao-Tsung Chang</td>
<td>103年8月16日 to 103年12月10日</td>
<td>莊偉哲 教授</td>
</tr>
</tbody>
</table>

### Development of anti-hepatoma drugs using integrin antagonists

Flow cytometry was used to determine integrins αv, β1, α5β1, αvβ3, αvβ5, αvβ6, and α2β1 expression levels of HCC cell lines (HuH7, Hep3B, HepG2, and PLC) and HSC. Our analysis has shown that integrins αv and β1 were highly expressed in all of the hepatoma cells, and expression levels of integrins α5β1, αvβ3, αvβ5, αvβ6, and α2β1 on HCC cell lines were very different. In contrast, integrin expression levels on HSC were very low. These results suggested that integrins αv and β1 may be important for HCC therapy.

![Integrin expression profiles of HCC cell lines and HSC](image)

**Integrin expression profiles of HCC cell lines and HSC**

This figure shows the expression levels of integrins on different cell lines. The solid peak represents the IgG control, the hollow peak is the signal of integrin antibodies, and the white peak moves to the right, indicating a higher expression level in the cell itself.
Inhibition of hepatoma cell adhesion by disintegrins

Dr. Chuang’s lab has successfully designed integrin αvβ3-specific (ARLDDL) and αvβx and α5β1-specific (KG and KG-P) disintegrins. In this study, we determined the inhibition of cell adhesion of HCC cells using these disintegrins. The adhesions of hepatoma cells to fibronectin and vitronectin are correlated with the expression levels of integrins αvβ3 and α5β1, as well as integrins αvβ3 and αvβ5, respectively. The results showed that Rho, KG and KG-P significantly inhibited the adhesion of hepatoma cells (HuH7, Hep3B, and HepG2) to fibronectin and vitronectin, and exhibited higher activity in inhibiting the adhesion to vitronectin. In contrast, ARLDDL weakly inhibited hepatoma cell adhesion to fibronectin and vitronectin due to its lower integrins α5β1 and αvβ5 inhibitory activities. Because Rho has higher integrins αvβ3 and αvβ5 inhibitory activity than KG and KG-P, it exhibited highest activity in inhibiting cell adhesion to vitronectin. Interestingly, we found that integrins-specific disintegrins weakly inhibited PLC cell adhesions to fibronectin and to vitronectin because integrins α5β1 and αvβ5 were highly expressed in PLC cells. These findings were consistent with previous reports that hepatoma cell adhesions to fibronectin and to vitronectin are mainly regulated by integrin α5β1, and integrins αvβ1 and αvβ5, respectively (Nejjari et al., 2002).
**圖 3. 肝癌細胞黏附 fibronectin 與 vitronectin 能力**

A. HuH7、Hep3B、HepG2 與 PLC 細胞黏附能力較強，Hep3B+HBx 細胞較弱。
B. HuH7、Hep3B 與 PLC 細胞黏附能力較強，Hep3B+HBx 及 HepG2 細胞較弱。

平均數值以 means ± standard error of mean 呈現。***表示 p-value 小於 0.001。

<table>
<thead>
<tr>
<th></th>
<th>HuH7</th>
<th>Hep3B</th>
<th>Hep3B+HBx</th>
<th>HepG2</th>
<th>PLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rho</td>
<td>569.9 ± 181.4 (3)</td>
<td>1136.7 ± 135.3 (3)</td>
<td>ND</td>
<td>ND</td>
<td>&gt;54948.3 (41.9%)</td>
</tr>
<tr>
<td>ARLDDL</td>
<td>&gt;68043.0 (44.5%)</td>
<td>&gt;68043.0 (33.3%)</td>
<td>ND</td>
<td>ND</td>
<td>&gt;33761.8 (37.4%)</td>
</tr>
<tr>
<td>KG</td>
<td>65.3 ± 4.0 (3)</td>
<td>53.0 ± 9.2 (3)</td>
<td>ND</td>
<td>ND</td>
<td>9162.3 ± 4004.4 (3)</td>
</tr>
<tr>
<td>KG-P</td>
<td>57.3 ± 13.2 (3)</td>
<td>77.9 ± 32.7 (3)</td>
<td>ND</td>
<td>ND</td>
<td>35823.0 ± 16641.1 (2)</td>
</tr>
</tbody>
</table>

ND: Non-detectable，細胞黏附少

**表 1. 去整合蛋白對肝癌細胞黏附 fibronectin 的抑制濃度**

KG、KG-P 相對於 Rho 有較好的抑制效果，ARLDDL 則更差。Hep3B+HBx 與 HepG2 細胞由於黏附 fibronectin 能力差，無法進行抑制的實驗。平均數值以 means ± standard error of mean 呈現。

<table>
<thead>
<tr>
<th></th>
<th>HuH7</th>
<th>Hep3B</th>
<th>Hep3B+HBx</th>
<th>HepG2</th>
<th>PLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rho</td>
<td>7.6 ± 2.6 (3)</td>
<td>29.4 ± 5.7(3)</td>
<td>13.6 ± 5.0 (3)</td>
<td>ND</td>
<td>224.1 ± 50.9 (3)</td>
</tr>
<tr>
<td>ARLDDL</td>
<td>132.4 ± 27.1(3)</td>
<td>355.3 ± 142.0(3)</td>
<td>ND</td>
<td>105.0 ± 27.5(3)</td>
<td>4444.0</td>
</tr>
<tr>
<td>KG</td>
<td>15.7 ± 0.4 (3)</td>
<td>43.5 ± 12.3(3)</td>
<td>ND</td>
<td>27.0 ± 3.1(3)</td>
<td>237.4 ± 46.6 (3)</td>
</tr>
<tr>
<td>KG-P</td>
<td>16.4 ± 2.1 (3)</td>
<td>23.4 ± 0.7(3)</td>
<td>ND</td>
<td>39.1 ± 5.1(3)</td>
<td>249.1 ± 14.1(3)</td>
</tr>
</tbody>
</table>

**表 2. 去整合蛋白對肝癌細胞黏附 vitronectin 的抑制濃度**

Rho、KG、KG-P 相對於 ARLDDL 有較好的抑制效果。Hep3B+HBx 細胞由於黏附 vitronectin 能力差，無法進行抑制的實驗。平均數值以 means ± standard error of mean 呈現。
二、研究或教學或科技研發與管理成效評估（由計畫主持人或單位主管填寫）

Please evaluate the performance of research, teaching or science and technology R&D and management work: (To be completed by Project Investigator or Head of Department/Center)

(1) 是否達到延攬預期目標？

Has the expected goal of recruitment been achieved?

Yes, the goal has been achieved.

(2) 研究或教學或科技研發與管理的方法、專業知識及進度如何？

What are the methods, professional knowledge, and progress of the research, teaching, or R&D and management work?

The research is on schedule.

(3) 受延攬人之研究或教學或科技研發與管理成果對該計畫(或貴單位)助益如何？

How have the research, teaching, or R&D and management results of the employed person given benefit to the project (or your unit)?

Dr. Chang is excellent in protein engineering, protein structural analysis, and cell manipulation. He has set up the standard operating procedure for the cell adhesion assays and protein crystallization. Thanks to his hard work and effort, our lab can settle into a routine.

(4) 受延攬人於補助期間對貴單位或國內相關學術科技領域助益如何？

How has the employed person, during his or her term of employment, benefited your unit or the relevant domestic academic field?

These results from Dr. Chang elucidated that disintegrins can inhibit the adhesion of hepatoma cell to fibronectin and vitronectin. This study will serve as the basis on development of the potent integrin-specific drugs for the treatment of hepatocellular carcinoma.

(5) 具體工作績效或研究或教學或科技研發與管理成果：

Please describe the specific work performance, or the results of research, teaching, or R&D and management work:

Dr. Chang has done a lot of research on the cell-based assay and functional assay. Followings are main achievement:

1. Flow cytometric analysis showed that integrins αv and β1 were ubiquitously expressed in all of the hepatoma cells. In contrast, integrin expression levels were low on hepatic stellate cell.

2. The expression of HBx in Hep3B cell (Hep3B+HBx) caused 3- and 1.5-fold decreases in the expression of integrins α5β1 and αvβ3 in comparison with those of Hep3B, resulting in the decreases in hepatoma cell adhesion to fibronectin and vitronectin.

3. Cell adhesion analyses showed that multiple integrins-specific disintegrins, Rho, KG and KG-P, significantly inhibited the adhesion of hepatoma cells (HuH7, Hep3B, and HepG2) to fibronectin and vitronectin, and exhibited higher activity in inhibiting the adhesion to vitronectin. In contrast, ARLDDL, an integrin αvβ3-specific disintegrin, weakly inhibited hepatoma cell adhesion to fibronectin and vitronectin.

(6) 是否續聘受聘人？ Will you continue hiring the employed person?  □ 繼聘 Yes  ■ 不續聘 No

Dr. Chang is going to do the substitute military service.

※此報告表篇幅以三～四頁為原則。This report form should be limited to 3-4 pages in principle.

※此表格可上延攬優秀人才成果報告繳交說明網頁下載。This report form can be downloaded in http://scholar.lib.ncku.edu.tw/explain/