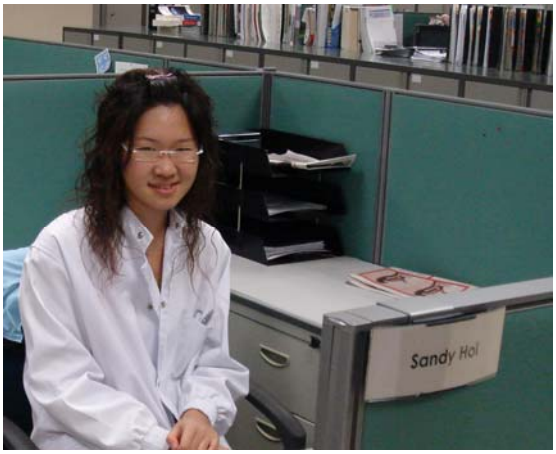
The Chinese University of Hong Kong
Department of Biochemistry
Summer Internship Program in
Technology & Product Development of
CK Life Sciences Ltd.

Name: Hoi Wan Heng, Sandy
SID: 03503224
Period of employment: 3/8 to 31/8/2006
Supervisor: Dr. Eric Xing

Background

The company is screening a series of chemicals to choose candidates for pharmaceutical significance. These chemicals may be synthesized chemically or extracted from herbs. In the screening process, the candidates have to be treated with different cell lines. Those responsive cell lines would be chosen for IC50 determination, followed by tests for certain diseases. After passing through all these pre-clinical stages, the candidates would be subject to clinical trials. A drug may be produced finally, and their qualities would be monitored and controlled.



I participated in the pre-clinical screening of two drugs, namely 5601 and 5605, and we have to determine their cytotoxic effects and hemoglobin induction on K562 cells. K562 is a human erythroleukemia suspension cell line which can be induced to differentiate along the erythroid lineage by different chemicals.

Experimental procedures

Alamar Blue Assay

5601 and 5605 are candidates for treating sickle cell anemia.

We treated 5601 and 5605 with K562 cells and determine their IC50s with the alamar blue assay. The assay aims at estimating the cell density since alamar blue would be reduced by the redox reactions of cells. It was found that 5605 has a smaller IC50 than 5601 and thus is more toxic to K562. With their estimated IC50 values, we set the ranges of their concentrations for hemoglobin induction assay.

Hemoglobin Induction Assay

A well-known drug for treating sickle-cell disease patients was used as the positive control for our assay. It induces gamma-globin expression in human erythroid progenitor cells and thus increases fetal hemoglobin (HbF) production. This reduces hemoglobin S polymerization and clinical complications of patients. We would like to know if our drugs could induce hemoglobin expression in K562 cells.

The drugs were treated with K562 for 2-3 days, and the cells were washed with PBS, and standardized to the same concentration. After lysing the cells, TMB staining solution was added, and its absorbance was measured after 10 minutes incubation. The hemoglobin content was calculated with a hemoglobin standard curve. The result showed that 5605 is more potent in hemoglobin induction. Besides, we found that the quantity of hemoglobin produced after 3

days of drug treatment is greater than that after 2 days. For the positive control, there is no significant difference between the amounts of Hb produced after the two incubation time. Due to time limitation, the assay with the same condition was not repeated, and therefore I'm not able to make more precise conclusions with statistic methods.

Conclusion and future studies

However, we are not able to conclude if the drugs are able to cure sickle-cell disease at this stage. It is because we do not know if the hemoglobin produced are HbF, which is the only effective hemoglobin form in relieving symptoms in sickle-cell disease patients. Therefore, RNA analysis should be carried out following the hemoglobin assay. The RNAs coded for gamma-globin should be quantified to determine if that gene is up-regulated. Due to time limitation, I did not take part in that assay.

Final remarks

Through the program I had acquired knowledge on simple cell culture and cell treatment techniques. It also gave me a rough idea on drug screening and development, and how a drug company operates under the co-ordinations of different departments. My supervisor and all the staff were willing to teach me a lot. It was an invaluable opportunity for me to have some ideas on the difference between working and studying, and it also inspired my interests and confidence in scientific research.

End of Report

Date: 31/8/2006