

Peptide deformylase (PDF) is essential and unique to bacteria for cytoplasmic protein synthesis, but not required in eukaryotes, thus making it an attractive target for the discovery of novel antibacterial drugs. Protein synthesis in eubacteria, under normal conditions, is initiated by formyl-methionyl-tRNA. PDF removes the formyl-group of N-formylmethionine of newly synthesized polypeptides to produce a mature protein. In this study, a pdf gene from *Staphylococcus aureus* 6538p was cloned in pET-14b vector and transformed in *Escherichia coli* BL21 (DE3). PDF protein was overexpressed by addition Isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG). NH<sub>2</sub>-terminal His-tagged PDF protein was purified by nickel-nitrilotriacetic acid (Ni-NTA) metal-affinity chromatography. Enzymatic activity of purified 6xHis-tagged PDF was tested on the substrate, formyl-Methionine-Alanine-Serine (fMAS), by formate dehydrogenase-coupled spectrometric assay of peptide deformylase. For the discovery of new PDF inhibitors from chemical libraries and culture broths of soil bacteria, a target-oriented screening system using a 96-well plate was developed. About 50,000 commercial chemical libraries were tested in this screening system, and about 100 chemicals (0.2 %) among them showed an inhibitory activity against PDF enzyme. This result shows that a new screening system can be used for the discovery of new PDF inhibitors.

[PC3-1] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Anti-angiogenic activity of wilfoside glycosides isolated from *Cynanchum wilfordii***

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Wm was isolated from *Cynanchum wilfordii* (Asclepiadaceae) as a mixture form of polypregnane glycosides that included wilfoside K1N and wilfoside C1N. In the present study, we investigated the anti-angiogenic effect of wilfoside glycosides using in vivo and in vitro assay systems. We first demonstrated that concentrated conditioned media obtained from Wm-treated HepG2 human hepatoblastoma cells blocked the angiogenic activation of Wm-untreated concentrated conditioned media, suggesting that Wm may have an inhibitory effect on tumor-induced angiogenesis. In addition, Wm decreased both neovascularization of chick embryos in the chorioallantoic membrane assay and basic fibroblast growth factor-induced vessel formation in the mouse Matrigel plug assay. Interestingly, the angiogenesis inhibition of Wm was the most dramatic in comparison with those of wilfoside K1N and wilfoside C1N, indicating that wilfoside K1N and wilfoside C1N may have stronger effects when they are co-treated in a mixture form. Moreover, wilfoside K1N reduced tube formation and proliferation of human umbilical vein endothelial cells. Taken together, our present study suggests that wilfoside glycosides may be strong angiogenic inhibitors with a potential of therapeutic application on hypervascularizing tumor cells.

[PC3-2] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **In vitro neural differentiation of human embryonic stem cells**

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Human embryonic stem (ES) cell lines derived from the inner cell mass of human blastocysts have potential to differentiate into any cell types. We have established in vitro neural differentiation of human ES cells. After the formation of embryoid bodies (EBs), the differentiating EBs formed neural tube-like rosettes in the presence of basic fibroblast growth factor (bFGF). The rosettes were selectively isolated by the treatment of dispase and cultured in a medium for human neural precursors in the presence of bFGF. Finally, after the human neural precursors were cultured in the absence of bFGF, the neural precursors differentiated into three neural lineages, neurons, astrocytes, and oligodendrocytes. The in vitro neural differentiation of human ES cells provides a powerful tool for both basic neuroscience research and therapeutic application.