

Sirigade Ruekit 2009: Development of an Inhibiting Activity Assay of Tyrosine Kinase of EGFR for Screening the New EGFR Inhibitor. Master of Science (Biochemistry), Major Field: Biochemistry, Department of Biochemistry. Thesis Advisor: Assistant Professor Kiattawee Choowongkomon, Ph.D. 160 pages.

EGFR regulates a wide range of cellular processes. For these reasons, EGFR became a target protein for cancer therapy. Herbs have been considered valuable sources for anti-cancer drug discovery. In our studies, we tried to develop the method for activity assay of tyrosine kinase (TK) of EGFR in order to screening for new EGFR inhibitors from Thai herbs. These included the suitable host for TK expression and comparing the suitable activity assay for testing TK inhibitors. We compared the expression of TK protein in both bacterial and insect expression systems. From bacterial expression system, The non-fusion TK protein had no activity but GST-TK that expressed at 16°C had a weak activity (lower V_{max} and higher K_m values). The final yield of purified GST-TK was 7.7 mg per liter of culture. Due to the lower yield and weak activity of TK in bacterial cells, we turned to use the insect expression system. The final yield of purified TK and GST-TK in insect expression systems were 6.74 mg protein/15 ml culture medium and 6.06 mg protein/15 ml culture medium, respectively. The K_m and V_{max} of GST-TK was similar to the commercial full-length EGFR. Moreover, recombinant TK has a high degree of glycosylation while recombinant GST-TK has no glycosylation at all. Thus, the suitable system for expression of active recombinant TK is using the insect cells expression system by expressing as the fusion GST-TK. For developing activity assays of TK, we used three methods for comparing efficiencies; ABTK assay, ATP/NADH couple assay and PURETIME assay. The ABTK assay had sensitivity and specificity more than other methods. Then we used ABTK assay for screening inhibitor from Thai herbs. The crude ethanol extracts from 25 Thai herbal plants were used in this experiment. The results showed that the crude extraction of *S. indicum* and *M. acochinchinensis*, *T. chebula*, *C. loureiroi* and *Ruellia tuberosa* had inhibition activity against TK of EGFR. This is the first report of the identification of Thai herbal extracts which can inhibit EGFR kinase activity by using molecular biology techniques combined with enzyme kinetic studies. These findings may lead to the further development of novel anti-cancer drugs.

Student's signature

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