計畫編號：NSC 88-2314-B006-122
非小細胞肺癌細胞株中 p130-Rb2 的可能表現
執行期限：87 年 7 月 1 日至 88 年 7 月 31 日
主持人：賴吾為 國立成功大學醫學院 附設醫院 外科部 胸腔外科
中文摘要:
目的：腫瘤抑制蛋白Rb家族中的p130藉著兩種作用方式抑制細胞的分裂。一是和E2Fs（E2F4）結合，讓細胞處於不分裂狀態。二是和cyclin E結合，尤其在fibroblast中，抑制cyclin E-associated kinase。我們想探討p130在肺癌細胞株是否有降低的現象。若有降低的話，是不是可以當作肺癌惡性度的指標之一。
結果：經一連串IB實驗，發現H520和H125這兩種肺癌細胞株中cyclin E的含量明顯高於正常氣管上皮細胞株（Bes2B），但cdk2的含量僅是略高。而Rb-p130卻一度沒有辦法測出來。另外嘗試用immunoprecipitation（IP）來測這些株肺癌細胞株cyclin E kinase activity的高低，也是碰到瓶頸，重覆多次一直得不到結果。
結論：目前得到一些初步結論是某些肺癌細胞株（H520, H125）中的cyclin E的量確實比正常氣管上皮細胞株（Bes2B）來的高。因一些技術上的瓶頸仍待突破中，所以這種現象的背後含意仍待探索中。
Abstract:

Purpose: We are going to explore the role of Rb-p130 in the pathogenesis of non-small cell lung cancer. The level of Rb-p130 was compared between the immortalized normal bronchial epithelial cell line and three non-small cell lung cancer cell lines.

Material and Methods: The cell lines we used were as follow: 1. Immortalized normal bronchial epithelial cell lines (Bes2B) used as control group of this experiment. 2. Non-small cell lung cancer cell lines, H460 (wild p53), H125 (mutant p53), H520 (wild p53) used as experimental group. 3. Leukemia cell line – manca cell line use as cyclin/cdk positive control. We used immunoblot to detecte the level of cyclin E, ckd2, Rb-p130, cyclin D and p27 in the above cell lines. The immunoprecipitation was used to evaluate cyclin E-associated kinase activity. We will use IP/westren with anti-cyclin E Ab to determine whether cycline E associate with p130 or with CKI (cdk inhibitors).

Result: The result of IB shown the level of cycline E of H520 and H125 was higher than that of Bes2B. The level of cdk2 was slight higher only. The level of Rb-130 could not be shown by the method of IB. We failed to demonstrate the cyclin E-associated kinase activity by the method of IP.

Conclusion: The temporary conclusion we made was the level of cyclin E of H520 and H125 was higher than that of normal bronchial epithelial cell line. Since we struggled with IB of Rb-p130 and IP of cyclin E-associated kinase activity, the real role of elevated cyclin E in the pathogenesis of non-small cell lung cancer cell need to be further explored.
Introduction:

The cyclin-dependent kinase and their associated regulatory cyclins control the progression of cell cycle and cell growth. A loss of cell-cycle control may contribute to tumor formation.

P130, a Rb-related protein, may suppress proliferation in two ways: First, it interacts with a subset of E2Fs (E2F4), and may have roles in maintaining cells in a non-proliferating state. In addition, in fibroblast, interacts between p130 and cyclin E had been documented and suggests that this interaction inhibits the cyclin E-associated kinase. Consequently, the loss of p130 could effect the signals that drive cells out of cycle, both by E2F mediated transcription and by direct interference with cdk2 activity. We would like to prove whether the p130 is a good prognostic marker in NSCLC or not?

The cell lines we used were as follow: 1. Immortalized normal bronchial epithelial cell lines (Bes2B) used as control group of this experiment. 2. Non-small cell lung cancer cell lines, H460 (wild p53), H125 (mutant p53), H520 (wild p53) used as experimental group. 3. Leukemia cell line – manca cell line use as cyclin/cdk positive control.

We would like to check the cyclin E-associated kinase activity of the cell lines mentioned above by the method of immunoprecipitation H1 kinase assay. Next, does this kinase activity correlate with an increase in cyclin E protein? We will try to prove it with the method of immunoblot. The expression of CDK inhibitors (CKI; p21, p27, p57), the Rb-related protein p130 and E2F4 will be checked by immunoblot. In addition, determine if cyclin E associates with p130 and the CKI by IP/western with anti-cyclin E antibodies (IP+IB).

The result of IB shown the level of cycline E of H520 and H125 was higher than that of Bes2B. The level of cdk2 was slight higher only. The level of Rb-130 could not be shown by the method of IB. We failed to demonstrate the cyclin E-associated kinase activity by the method of IP.

The temporary conclusion we made was the level of cyclin E of H520 and H125 was higher than that of normal bronchial epithelial cell line. Since we struggled with IB of Rb-p130 and IP of cyclin E-associated kinase activity, the real role of elevated cyclin E in the pathogenesis of non-small cell lung cancer cell need to be further explored.
References:


