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| 摘要(中) | 肺癌，是台灣與當前全世界癌症人口的主要死亡原因，因此，探討肺癌的病理機轉在治療或偵測肺癌將有所助益。利用基因微陣列技術（microarray）大規模分析肺腺癌患者腫瘤（tumor part）與其鄰近正常組織（adjacent normal part）中基因的表現差異時，我們發現了一個新的基因，FLJ20152，在腫瘤組織的表現量低於鄰近正常組織（$p < 0.0001$）；定量 RT-PCR 進一步證實 FLJ20152 mRNA 在腫瘤組織的確有較低的表現量（$p = 0.002$），且此基因的位置座落於 LOH（loss of heterozygosity）區域附近，故推測其可能與抑制腫瘤的產生有關。為確定腫瘤組織中 FLJ20152 基因是否發生突變，我針對 15 個肺癌患者腫瘤組織及 13 個肺癌細胞株的基因體，將會轉譯為 FLJ20152 蛋白質的核?酸序列（coding sequence）做定序。目前，我並沒有找到任何會造成胺基酸改變的突變（non-synonymous mutation），只分別在 exon 6（T180T/C）及 exon 8（C393C/T 或 C393T）各找到一個不會造成胺基酸改變的突變（synonymous mutation），且存在於 NCBI 的 SNP（single nucleotide polymorphism）資料庫中，這說明肺癌的形成可能不是因為 FLJ20152 基因突變所導致。本論文中，我首先建立 FLJ20152 蛋白質的酵母菌雙合雜交系統（yeast two-hybrid system），期藉此找到與其發生交互作用（protein-protein interaction）的蛋白質，以推測 FLJ20152 的功能。目前，我找到 8 個可能會與 FLJ20152 發生交互作用的蛋白質，包括 THY1、MAGED4、OLFML3、ATP1B1、NUDC、P2Y5、BF 及 ATP1A1，其中 ATPA1 與 THY1 已被證實會由不同的路徑調控細胞增殖，不受控制的細胞增殖為癌症形成的特色之一，FLJ20152 與這兩個蛋白質的交互作用意謂著其可能可以影響細胞的生長。如上述，在分析可持續表現 FLJ20152 蛋白質的肺癌細胞株 H1299 在細胞週期的分布，與對照組做比較後，發現 G0/G1 期的細胞數增加的趨勢，顯示 FLJ20152 蛋白質可能確實在 G1/S 期的行進上佔有一定的角色。另外，我發現 FLJ20152 蛋白質以點狀分布於細胞質中。然而，FLJ20152 的分布與功能及其在癌症組織低量表現關聯為何仍有待更多的實驗結果來證實。本論文中，我使用 shRNA 及 siRNA 抑制 FLJ20152 基因的表現，並製備 GST-FLJ20152 融合蛋白質用以製作抗體。上述研究將為後續 FLJ20152 研究的基礎以利我們對 FLJ20152 功能及其與肺癌形成關聯做進一步探討。 |
| 摘要(英) | Lung cancer is currently the leading cause of cancer death worldwide as well as in Taiwan. Identifying the causal perturbations that confer lung carcinogenesis may significantly advance the detection and the treatment of cancer. Recently, we have analyzed the lung adenocarcinoma by Affymetrix microarray profiling, and proposed to investigate one novel gene, FLJ20152, by its' down-regulation in lung cancer tissue as compared to adjacent normal part ($p < 0.0001$) and its positioned near LOH region. Using Q-RT-PCR analysis, we also validate the expression of FLJ20152 mRNA is indeed lower in lung tumor tissues ($p = 0.002$), indicating it may at least, |

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| | in part, plays roles on tumor suppression. In attempt to know whether FLJ20152 gene was mutated in tumor tissues, the coding sequences of FLJ20152 in 15 lung cancer patients and 13 lung cancer lines were sequenced. So far, I didn’ t find any non-synonymous mutations in tumor tissues. Only one heterozygous synonymous mutation on exon 6 (T180T/C) and one synonymous mutation on exon 8 (C393C/T or C393T), which also be mentioned in NCBI SNP database were found, indicating no mutation of FLJ20152 was obviously linked to lung cancer formation. To elucidate the role of FLJ20152, I firstly established a yeast two-hybrid system to identify its interacting protein(s). I have identified eight proteins were shown to interact with FLJ20152 including THY1, MAGED4, OLFML3, ATP1B1, NUDC, P2Y5, BF and ATP1A1. Among these candidate interacting proteins, ATP1A1 and THY1 were shown to regulate cell proliferation by different ways, indicating the interacting of them and FLJ20152 may also play roles in regulating cell growth, which is one of the characteristics of cancer formation. As indicated above, FLJ20152 stably expressed H1299 cells show increase of G0/G1 phase cells as compared to the control cells, indicating this protein might indeed play a role in G1/S progression. I have found that FLJ20152 displays tiny speckled morphology within cytoplasm, however, how it functions and corrects with its down-regulation in lung cancer cells still to be further resolved. In the present thesis, I have used shRNA and siRNA for knock-down the expression of FLJ20152, generate the GST-FLJ20152 fusion protein for immunizing animals, which will form the basis for our further studying the function of FLJ20152 and its link in lung cancer formation. |
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