**ABSTRACT**

Lung cancer is the most common cause of cancer death in the world, and lung cancer patients death mainly due to metastasis. The process of metastasis have many complex mechanisms involved, consist of many different functional genes. The main purpose of this study is screening with lung cancer metastasis-related genes, and thus further explore the molecular mechanisms of cancer metastasis. Previously, using microarray assay and lung cancer metastasis model cell lines, screening with cancer metastasis-related genes. Additionally, we focus on cancer metastasis-related gene, Desmocollin-2. In this study, Desmocollin-2 expression in highly metastatic lung cancer cell lines (such as A549 and CL1-5) is fewer than low metastatic lung cancer cell (CL1-0), these result have proved that Desmocollin-2 gene expression levels in lung cancer cells were negatively correlation with the metastasis activity of a panel of lung cancer cell lines. Using shRNA-DSC2 transfection methods, we suggested that knockdown DSC2 gene expression can promote lung cancer cells proliferation and migration, at the same time inhibit Desmocollin-2 gene expression also promote lung cancer cells colonyforming. Finally found that stable suppression Desmocollin-2 gene in cells whose morphology similar Epithelial to mesenchymal transition (EMT) phenomenon, These result have proved that Desmocollin-2 may play a role in tumor suppressor.
4.11 質體DNA萃取

4.11.1 傳統法質體DNA萃取

4.11.2 質體DNA之kit萃取

4.12 DNA電泳

4.13 DNA膠體kit純化

4.14 MTT assay

4.15 Colony formation assay

4.16 細胞遷移分析 (Cell migration assay)

4.16.1 wound healing assay

4.16.2 Transwell migration assay

4.17 西方墨點分析 (Western Blot)

4.17.1 SDS膠體的製備

4.17.2 蛋白質樣品製備

4.17.3 蛋白質的定量

4.17.4 SDS膠體電泳

4.17.5 電轉

4.17.6 抗體雜合

4. 結果

4.1 透過程microarry assay的分析比較CL1-5以及CL1-0細胞中的基因表現情形

4.2 藉由即時定量PCR的方式分析CL1-5以及CL1-0肺癌細胞中的基因表現情形

4.3 藉由即時定量PCR的方式分析Desmocollin-2-ab在侵入能力不同的細胞株中mRNA表現量的差異

4.4 藉由即時定量PCR的方式分析不同的細胞株中表現量的差異

4.5 以西方墨點法分析Desmocollin-2在侵入能力不同的肺癌細胞株中蛋白質表現量的差異

4.6 以即時定量PCR的方式分析shDSC2的抑制效果

4.7 以wound healing assay來分析其Transient transfection shDSC2-497、pGIPZ的CL1-0細胞的移動能力

4.8 以colony formation assay分析Transient transfection shDSC2、pGIPZ的CL1-0細胞群落形成的能力

4.9 以MTT assay分析Transient transfection shDSC2、pGIPZ的CL1-0細胞的增生速度

4.10 以即時定量PCR的方式檢測轉染shDSC2的CL1-0細胞株

4.11 以西方墨點法分析穩定抑制Desmocollin-2基因的細胞株

4.12 以transwell migration分析穩定抑制Desmocollin-2基因的CL1-0細胞株

4.13 以colony formation assay分析穩定抑制Desmocollin-2基因的CL1-0細胞株其細胞群落形成的能力

4.14 以Western Blot法分析穩定抑制Desmocollin-2基因的CL1-0細胞株增生的速度

4.15 以Western Blot法分析穩定抑制Desmocollin-2基因的CL1-0細胞株增生的情形

5. 結論

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