探討轉化生長因子 β 受體 2 對橋粒芯蛋白 2 在口腔鱗狀細胞癌中的調節作用及對於細胞遷移的影響

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口腔癌為頭頸癌中的一種，是全球十大癌症之一，在台灣男性十大癌症死亡率當中也位居第四位。口腔癌中有90%的患者罹患的為口腔鱗狀細胞癌(Oral Squamous Cell Carcinoma；OSCC)，主要造成的緣由有長期嚼食檳榔、抽菸、飲酒及人類乳突病毒的感染，而台灣患者其致病原因多數為長期嚼食檳榔、抽菸及飲酒。造成口腔癌患者病情惡化進入癌症病期三或四期的原因為癌細胞的轉移，而癌細胞轉移到其他部位也造成了治療困難，使五年存活率低下。因此能在早期就能檢測並藉由手術、化療等方式進行治療就顯得相當重要。

第二型橋粒醣蛋白(desmoglein 2, Dsg2)是組成橋粒(desmosome)的重要蛋白之一，負責細胞之間的黏附。Dsg2在頭頸癌中有著雙面的作用，一方面Dsg2可以協助癌細胞之間的黏合，增加癌組織的體積，也可以協助外泌體的釋放，刺激癌細胞之間的增生及轉移。而另一方面，也有研究指出Dsg2表現量的減少會使聚集的腫瘤細胞之間的黏附力降低而分散出去。因此Dsg2表現量的變化是判斷癌細胞是否進行上皮-間質轉化(EMT)的指標之一。第二型乙型轉化生長因子受器(transforming growth factor beta receptor 2, TGFBR2)是乙型轉化生長因子(TGF-beta)的主要受器之一。而TGF-beta在頭頸癌中也有著雙面作用，一方面可以停滯細胞週期，抑制癌細胞增生，而另一方面也可以透過與TGFBR2的作用使癌細胞進行上皮-間質轉化使癌細胞更容易擴散出去。但是Dsg2和TGFBR2在頭頸癌或口腔癌中的作用仍然不清楚。近期發表於科學期刊(Science)的一篇研究顯示，透過系統生物學的方式發現Dsg2和TGFBR2可能有蛋白之間的交互作用，而此作用可能和細胞傾向正常或癌化有關，但此作用和關係目前並無相關研究證明。因此，我們透過西方墨點法及即時聚合酶連鎖反應檢視並比較口腔接近正常細胞株(SG)、分化自日本舌癌病患細胞株(SAS)及台灣口腔癌患者所建立二株細胞株(OC2，OCSL)中Dsg2和TGFBR2的蛋白質及RNA表現量是否有差異。而目前有發現較為惡性的OCSL和OC2相比其DSG2和TGFBR2的蛋白質表現量都比較高。而未來也會也以Pull-don assay以及Co-IP確認TGFBR2-Dsg2是否有無交互作用，再進一步找出交互作用的團域(domain)。此外，透過建構完成的質體基因轉殖的方式，讓Dsg2或TGFBR2的蛋白可大量表現或靜默，以MTT等測量方式以評估各口腔癌細胞株增生、轉移和入侵能力。這些細胞株會以空質體(empty plasmid)或是錯亂siRNA(scrambled siRNA)作為比較控制組。若證實此二蛋白的交互作用，將進一步驗證TGFBR2與Dsg2的交互作用之致癌(或抑癌)機轉與途徑。

Investigation of modulatory effects of transforming growth factor beta receptor 2 to desmoglein 2 in oral squamous cell carcinoma and its effect on cell migration

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Oral cancer is one of the head and neck cancers, one of the top ten cancers in the world, and even ranked as the fourth for male in Taiwan. 90% of patients with oral cancer are oral squamous cell carcinoma (Oral Squamous Cell Carcinoma; OSCC), which is mainly caused by long-term betel nut chewing, smoking, drinking and human papillomavirus infection. Most of the pathogenic causes of Taiwanese patients are long-term betel nut chewing, smoking and drinking. The reason for the deterioration of oral cancer patients into the third or fourth stage of cancer is the metastasis of cancer cells, and the metastasis of cancer cells to other parts also makes treatment difficult and the five-year survival rate is low. Therefore, it is very important to be able to detect it at an early stage and treat it by surgery, chemotherapy and other methods.

Desmoglein 2 (Dsg2) is one of the structure proteins of desmosome, which is responsible for the adhesion between cells. Dsg2 has a double-sided role in head and neck cancer. On the one hand, Dsg2 can assist the adhesion between cancer cells, increase the volume of cancer tissue, and can also assist in the release of exosomes to stimulate the proliferation and metastasis between cancer cells. On the other hand, some studies have pointed out that the reduction of the expression of Dsg2 will reduce the adhesion between the aggregated tumor cells and disperse them. Therefore, the change in the expression level of Dsg2 is one of the indicators for judging whether cancer cells undergo epithelial-mesenchymal transition (EMT). Transforming growth factor beta receptor 2 (TGFBR2) is one of the main receptors for beta-transforming growth factor (TGF-beta). TGF-beta also has a double-sided effect in head and neck

cancer. On the one hand, it can arrest the cell cycle and inhibit the proliferation of cancer cells, and on the other hand, it can also make cancer cells undergo EMT through the interaction with TGFBR2. easy to spread out. But the roles of Dsg2 and TGFBR2 in head and neck cancer or oral cancer remain unclear. A recent study published in the journal “Science” showed that Dsg2 and TGFBR2 may have a protein-to-protein interaction through systems biology, and this effect may be related to the tendency of cells to become normal or cancerous, but this effect is not There is currently no relevant research to prove the relationship. Therefore, we examined and compared the oral near-normal cell line (SG), differentiated from Japanese tongue cancer patient cell line (SAS) and two cell lines established from Taiwan oral cancer patient by Western blotting method and real-time polymerase chain reaction (OC2, OCSL), whether the protein and RNA expression levels of Dsg2 and TGFBR2 are different between these cell lines. At present, it is found that the more malignant OCSL and OC2 have higher protein expression levels than DSG2 and TGFBR2. In the future, Pull-don assay and Co-IP will also be used to confirm whether TGFBR2-Dsg2 interacts or not, and then further find out the interacting domain. In addition, the Dsg2 or TGFBR2 protein can be expressed or silenced in a large amount by means of the constructed plastid gene transfer, and the proliferation, metastasis and invasion ability of each oral cancer cell line can be evaluated by measurement methods such as MTT. These cell lines were compared with empty plasmid or scrambled siRNA. If the interaction between the two proteins is confirmed, the oncogenic (or tumor suppressor) mechanism and pathway of the interaction between TGFBR2 and Dsg2 will be further verified.