行政院國家科學委員會專題研究計畫成果報告

細胞黏附分子(Cadherin-Catenin)在甲狀腺癌組織表現的研究

Immunohistochemical Study of Cadherin-Catenin Complex on Thyroid Neoplastic Tissue

計畫編號：NSC 88-2314-B-002-297
執行期限：87 年 8 月 1 日至 88 年 7 月 31 日
主持人：黃實宏　　執行機構：國立台灣大學醫學院外科

一、 中文摘要

正常上皮細胞之間的 adherens junction，是靠 cadherin 分子互相結合及附著在其上的 actin filaments 細胞骨架，如此才能構成一個完整而有功能的 adherens junction。此構造與細胞生長，極化及訊息傳遞途徑有密切的關係。E-cadherin(135kD)是 cadherin 家族的一成員，屬於一種 Ca^{++}-dependent 的穿膜醣蛋白，大約由 700-750 個氨基酸所構成。完整的 cadherin 功能，需要靠細胞質內三種蛋白質α-catenin(102kD) 、 β-catenin(88kD) 及 γ-catenin (82kD)的聯繫，才能使 cytoskeleton 接上去。此複合體已被證明與癌症成因及惡性侵犯程度有密切關係。在本研究中，我們對正常甲狀腺組織、甲狀腺濾泡性結節、良性甲狀腺濾泡腺瘤及惡性甲狀腺濾泡癌作免疫螢光染色及免疫轉漬分析，來檢查 E-cadherin 以及α-、β-及γ-catenin 的表現。在正常甲狀腺組織、濾泡性結節、良性濾泡腺瘤，此 4 種分子皆正常分佈在細胞與細胞連接處，由免疫轉漬分析也可加以印證。在惡性濾泡癌，其 E-cadherin 及α-catenin 則與在正常細胞之分布無異，但在 10 個受檢的組織中，有 8 例無法染出β-catenin，而有 7 例無法染出γ-catenin。這些結果顯示，分化型甲狀腺癌之生成因與 E-cadherin 本身較無關係，而β-與γ-catenin 的存在與否，可能可以作為癌症診斷的分子標記。

關鍵詞：細胞黏附分子、甲狀腺癌

Abstract

E-cadherin is a member of the cadherin family that plays a major role in epithelial integrity and tumorogenesis. Catenins are a group of cytoplasmic proteins that regulate the intracellular anchorage of cadherin and are required for the linkage between cadherin and the actin cytoskeleton. Loss of E-cadherin contributes to the pathogenesis in tumor invasion and gives a poor prognosis. In order to investigate the adhesion property of intercellular junctions in thyroid tumors, expression of α-, β-, and γ-catenin should also be studied. A correlation between these molecular markers and malignancy would be useful as a preoperative diagnostic test for thyroid neoplasms. The expression of E-cadherin, α, β, and γ-catenin were studied in normal and neoplastic thyroid tissue by immunofluorescence microscopy and Western blot analysis. In the normal thyroid and in nodular goiter, and follicular adenoma, staining for E-cadherin, α-, β-, and γ-catenin was seen mainly at the lateral surface of epithelial cells in the follicle and the presence of these molecules was confirmed by Western blot analysis. Follicular carcinoma tissue stained positive for E-cadherin and α-catenin, but the results of β- and γ-catenin immunostaining were highly variable, with β-catenin being absent in most follicular carcinomas (8/10) and γ-catenin being absent in some follicular carcinomas (3/10). These results suggest that E-cadherin expression was not reduced during the pathogenesis of differentiated thyroid malignancies. Impairment of the cadherin-catenin complex at the cell junction may contribute to the malignant progression of differentiated thyroid neoplastic tissue.

Keywords: cadherin, catenins, thyroid carcinoma cell

二、 緣由與目的

The process of cancer invasion and metastasis consists of a complex series of sequential steps (1, 2). The initial step is the

dissociation of cancer cells from the primary tumor due to breakdown of the cell adhesion system, which includes integrins, selectins, CD44 and members of the cadherin family (3, 4). Cadherins are a family of functionally related transmembrane glycoproteins responsible for Ca^{2+}-dependent cell-cell adhesion, and the inactivation of other adhesion systems has little effect on cell-cell adhesion when cadherins are functioning normally (5). E-cadherin, one of the classical cadherins, has been shown to play an important role in epithelial integrity and tumorogenesis. It is frequently reduced or absent in various epithelial tumors, e.g. of the breast, colon, stomach, kidney, esophagus, head, and neck (6, 7, 8). An immuno- histochemical study showed E-cadherin to be a differentiation marker in thyroid malignancies (9) and to be associated with the clinical outcome as an independent prognostic marker (10). A recent study has shown that the linkage between E-cadherin and the actin filaments of the cytoskeleton is essential for strong cell-cell adhesion. This linkage occurs at the adherens junction and is mediated by many associated junctional undercoat proteins, including the catenins and vinculin. The catenins are a group of proteins, consisting of α-(102kDa), β-(88kDa), and γ-(82kDa) catenin, that interact with the intracellular domain of E-cadherin (11). Thyroid carcinomas can be subdivided by morphological and functional criteria into (a) well-differentiated carcinomas that mainly retain intercellular junctions and are generally weakly invasive, and (b) poorly-differentiated carcinomas that have fewer cell-cell junctions and are more invasive (12, 13). E-cadherin has been shown to be a differential marker in thyroid malignancy. In undifferentiated thyroid carcinomas, E-cadherin is present in low amounts or even absent (9). Well-differentiated thyroid carcinomas originating from follicular cells are classified as papillary or follicular and show varied intensities of E-cadherin immunoreactivity, despite the presence of increased E-cadherin mRNA in the follicular carcinomas (9). It has also been shown that the state of differentiation and degree of invasiveness of carcinomas can determine cancer prognosis. E-cadherin immunostaining has been suggested as an independent prognostic indicator for differentiated thyroid carcinomas (14, 15). In the thyroid, very little is known about intercellular adhesiveness. Although E-cadherin has been shown to be a differentiation marker in thyroid malignancy (9) and the loss of E-cadherin expression in differentiated thyroid carcinoma is an indicator of a poor prognosis (10), no conclusive data have been obtained about the adhesion mechanism at the cell-cell junction as regards α-, β-, and γ-catenin. In this study, we used both immunofluorescence microscopy and Western blot analysis to examine the expression of E-cadherin and α-, β-, and γ-catenin in normal thyroid tissue, benign thyroid tumor, and differentiated thyroid carcinoma to obtain a better insight into the intercellular adhesive properties of differentiated thyroid carcinomas. Ideally, the preoperative differential diagnosis between benign and malignant thyroid neoplasms, performed on fine-needle aspiration biopsy smears, might be improved if specific changes in these molecules could be readily detected.

三、結果與討論

In the present study, we examined the distribution of the cadherin-catenin complex by immunostaining and Western blot analysis of normal human thyroid tissue and neoplastic tissues with various degrees of invasiveness. Impairment of the E-cadherin-mediated cell-cell adhesion system has been reported to be a characteristic of malignantly transformed cells (7). Using immunohistochemical staining and Western blotting, reduced E-cadherin expression has been shown to be associated with tumor dedifferentiation (9), increased invasiveness and a high incidence of lymph node metastasis in a number of human carcinomas (18). Our data show no apparent difference in the E-cadherin immunostaining pattern of samples from normal thyroids (n=12), benign thyroid disorders (n=14, multinodular goiter and follicular adenomas), and follicular carcinomas (n=10). This observation does not correlate well with the reduced E-cadherin expression found in carcinomas of the head and neck, stomach, bladder, prostate and esophagus (18). E-cadherin is reported to be absent in anaplastic thyroid carcinoma, although

E-cadherin staining of various staining intensities has been described in papillary thyroid carcinoma and follicular thyroid carcinoma (9). These observations suggest a possible role for E-cadherin as an invasion suppressor molecule (19). The patients in this study initially presented with various stages of tumor growth and regional lymph-node metastasis. As an additional indicator of invasiveness, the histological tumor invasion pattern (capsule, vascular, or adjacent tissue) did not correlate with E-cadherin expression in this study. Linkage between E-cadherin and the actin filaments of the cytoskeleton is mandatory in order to have strong cell-cell adhesion and is mediated by many associated proteins, including catenins, vinculin, and actin (11). Decreased E-cadherin-mediated adhesiveness can result from downregulation of E-cadherin, defective catenins or biochemical modification of catenins (20). Cytoskeletal disconnection of the cadherin-catenin complex and phosphoryaltion decrease can occur in thyroid carcinoma as compared with that in normal and adenomatous tissue (21). Deletion of the α-catenin gene or mutation of the α-catenin protein has been reported in the human lung carcinoma cell line, PC9, despite high expression of E-cadherin (22). Our observations show that α-catenin is present in normal thyroid tissue, nodular goiter tissue, follicular adenoma and follicular carcinoma, suggesting it has a minimal role in carcinogenesis in well-differentiated thyroid carcinoma. β-catenin was not detected in 8/10 follicular carcinoma tissue samples, but was normally expressed in multinodular goiter and follicular adenoma cells. β-catenin is involved in cell adhesion, morphogenesis and intracellular signal transduction (23). Certain motility factors, including hepatocyte growth factor and epidermal growth factor, can induce tyrosine phosphorylation of β-catenin, which can lead to increased cell dissociation, motility and invasion (7). When associated with α-catenin, β-catenin can form distinct heteromeric complexes with adenomatous polyposis coli protein, which can act independently of the cadherin-catenin complex and function as central regulators of cell adhesion, cytoskeletal intereaction and tumor suppression in colorectal and gastric cancer (24). A truncated β-catenin was found in the human gastric carcinoma cell line, HSC-39, together with normal expression of E-cadherin, and α-, and γ-catenins (25). Our results demonstrate that low or zero amounts of β-catenin were found in most of the tested follicular thyroid carcinomas, with or without vascular invasion. Currently, we cannot determine whether the lack of β-catenin immunoreactivity is due to a genetic change or results from impairment of the transcription or translation processes, although the possibility that the lack of β-catenin immunoreactivity is due to an epitope change can not be excluded. This suggests that E-cadherin expression is not a single parameter of invasiveness properties and that decreased expression of β-catenin can serve as an indicator for a differential diagnosis between follicular adenoma and follicular carcinoma. This test can be easily performed in stamp smears, and the preoperative differential diagnosis between benign and malignant thyroid neoplasms, performed on fine-needle aspiration biopsy smears, could perhaps be strengthened by immunohistochemical staining for the cadherin-catenin complex, especially β-catenin. β-catenin or γ-catenin (plakoglobin) is the central molecule linking α-catenin to E-cadherin to form the two different cadherin-catenin complexes in the cell (10). An absence of γ-catenin has been reported in gastric cancer (26). Although γ-catenin was found to be absent in 3/5 carcinoma samples by Western analysis, it was shown to be normally expressed in 7/10 follicular thyroid carcinoma tissue samples by immunofluorescence staining. In summary, little is known about the regulation of E-cadherin expression and the mechanism by which the catenins alter the cell adhesion system and signal transduction pathway. Our study on thyroid cancer patients revealed that the absence of β-catenin and γ-catenin, with normal expression of E-cadherin and α-catenin, is highly suggestive of thyroid malignancy. Immunostaining for the cadherin-catenin complex could be suggestive as an useful parameter for the examination for the differential diagnosis of malignancy, which can be easily performed by routine fine needle aspiration and smear preparation.

四、計劃成果自評

Through execution of this project, we gain more insight into the intercellular properties of thyroid cancer cell which is thought to play impart role for cancer invasiveness and metastasis. The correlation between these molecular marker and malignancy can be derived from the analysis of these data, which in turn could provide vital information on differential diagnosis between benign and malignant thyroid neoplasms. Potentially, it could be performed on fine-needle aspiration biopsy smear preoperatively in clinical use. Technician involved in this project will learn most of the techniques used in the modern biological research and help the principal investigator in developing future project.

五、 參考文獻

1. Fidler IJ 1989 Origin and biology of cancer metastasis. Cytometry 10: 673- 680.
2. Nicolson GL 1988 Cancer metastasis: tumor cell and host organ properties important in metastasis to specific secondary sites. Biochim Biophys Acta 948: 175-224.
3. Coman DR 1947 Mechanism of the invasiveness of cancer. Science (Washington DC) 105: 347-348.
4. McCucheon M, Coman DR, Moore FB 1948 Adhesiveness of malignant cells in various human adenocarcinomas. Cancer (Phila) 1: 460-467.
5. Takeichi M 1990 Cadherins: a molecular family important in selective cell-cell adhesion. Annu Rev Biochem 59: 237- 252.
6. Lipponen P, Saarelainen E, Ji H, Aaltomaa S, and Syrjanen K 1994 Expression of E-cadherin (E-CD) as related to other prognostic factors and survival in breast cancer. J Pathol 174: 101-109.
7. Dorudi S, Sheffield JP, Poulsom R, Northover JM, Hart IR 1993 E-cadherin expression in colorectal cancer. An immunocytochemical and in situ hybridization study. Am J Pathol 142: 981-986.
8. Shiozaki H, Tahara H, Oka H, Miyata M, Kobayashi K, Tamura S, Iihara K, Doki Y, Hirano S, Takeichi M, Mori T 1991 Expression of immunoreactive E-cadherin adhesion molecules in human cancers. Am J Pathol 139: 17-23.
9. Brabant G, Hoang-Vu C, Cetin Y, Dralle H, Scheumann G, Molne J, Hansson G, Jansson S, Ericson LE, Nilsson M 1993 E-cadherin: A differentiation marker in thyroid malignancies. Cancer Res 53: 4987-4993.
10. von Wasielewski R, Rhein A, Werner M, Scheumann GF, Dralle H, Potter E, Brabant G, Georgii A 1997 Immunohistochemical detection of E-cadherin in differentiated thyroid carcinomas correlates with clinical outcome. Cancer Res 57: 2501-2507.
11. Ozawa M, Baribault H, Kemler R 1989 The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. EMBO J 8: 1711-1717.
12. Gabbert H, Wagner R, Moll R, Gerharz C-D 1985 Tumor dedifferentiation: An important step in tumor invasion. Clin Exp Metastasis 3: 257-279.
13. Weidner KM, Behrens J, Vandekerckhove J, Birchmeier W 1990 Scatter factor: Molecular characteristics and effect on the invasion of epithelial cells. J Cell Biol 111: 2097-2108.
14. Palestini N, Cappello N, Cottino F, Durando R, Vecchiato D, Rendine S, Abeatici S 1989 Multifactorial study of prognostic factors in differentiated thyroid carcinoma. Ital J Surg Sci 19: 137-144.
15. Akslen LA, Haldorsen T, Thoresen SO, Glattre E 1991 Survival and causes of death in thyroid cancer: a population- based study of 2479 cases from Norway. Cancer Res 51: 1234-1241.
16. Towbin H, Staehelin T, Gordon J 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Natl Acad Sci USA. 76: 4350-4354.
17. Fritz JD, Swartz DR, Greaser M L 1989 Factors affecting polyacrylamide gel electrophoresis and electroblotting of high molecular-weight myofibrillar proteins. Anal Biochem 180: 205-210.
18. Hedinger C, Williams ED, Sobin LH 1989 WHO histological classification of thyroid

tumor: a commentary on the second edition. Cancer 63: 908-911.
19. Tamura S, Shiozaki H, Miyata M, Kadowaki T, Inoue M, Matsui S, Iwazawa T, Takayama T, Takeichi M, Monden M 1996 Decreased E-cadherin expression is associated with haematogenous recurrence and poor prognosis in patients with squamous cell carcinoma of the oesophagus. Br J Surg 83: 1608-1614.
20. Takeichi M 1991 Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251: 1451-1455.
21. Tamura S 1997 The E-cadherin- mediated cell-cell adhesion system in human cancers. Br J Surg 84: 899-900.
22. Serini G, Trusolino L, Saggiorato E, Cremona O, De Rossi M, Angeli A, Orlandi F, Marchisio PC 1996 Changes in integrin and E-cadherin expression in neoplastic versus normal thyroid tissue. J Natl Cancer Inst 88:442-449.
23. Shimoyama Y, Nagafuchi A, Fijita S, Gotoh M, Takeichi M, Tsukita S, Hirohashi S 1992 Cadherin dysfunction in a human cancer cell line: possible involvement of loss of alpha-catenin expression in reduced cell-cell adhesiveness. Cancer Res 52: 5770- 5774.
24. Ilyas M, Tomlinson IPM, Rowan A, Pignatelli M, Bodmer WF 1997 β-catenin mutations in cell lines established from human colorectal cancers. Proc Natl Acad Sci USA. 94: 10330-10334.
25. Rubinfeld B, Souza B, Albert I, Munemitsu S, Polakis P 1995 The APC protein and E-cadherin form similar but independent complexes with alpha- catenin, beta-catenin, and plakoglobin. J Biol Chem 270: 5549-5555.
26. Kawanishi J, Kato J, Sasaki K, Fujii S, Watanabe N, Niitsu Y 1995 Loss of E-cadherin dependent cell-cell adhesion due to mutation of the β-catenin gene in a human cancer cell line, HSC-39. Mol Cell Biol 15:1175-1181.
27. Ochiai A, Akimoto S, Shimoyama Y, Nagafuchi A, Tsukita S, Hirohashi S 1994 Frequent loss of α-catenin expression in scirrhous carcinoma with scattered cell growth. Jpn J Cancer Res 85: 266-273.
28. Cowin P, Kapprell H-P, Franke WW, Tamkun J, Hynes RO 1986 Plakoglobin: a protein common to different kinds of intercellular adhering junctions. Cell 46: 1063-1073.