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理事長 中島 章 殿

研究室で撮影した本人のスナップ写真、及び発表論文のコピーを添付

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研究テーマ 胃癌細胞株におけるAPC-catenin 複合体，E-cadherin-catenin複合体の発現について

2．本年度の研究業績

(1) 学会・研究会等においての口頭発表 (有) ・ 無 (学会名・内容)

a. Immunoblot analysis of the adenomatous polyposis coli-catenin and the E-cadherin-catenin complexes in gastric cancer cell lines. Digestive Disease Week and the 100th Annual Meeting of the American Gastroenterological Association. May, 1999, Orlando, FL, USA

b. 胃癌細胞株におけるAPC-catenin 複合体，E-cadherin-catenin複合体の発現について 第71回日本胃癌学会総会 1999. 6 東京

c. Relationship between phenotypic expression and alterations of microsatellites linked to the APC gene in intramucosal carcinomas of the stomach. 5th China-Japan Joint Histochemistry Symposium. May, 2000, Shanghai, China

(2) 学会誌等に発表した論文 有 ・ (無) (雑誌名・論文名)

3．今後の研究計画

今年度は　胃癌細胞株を用いて，胃癌細胞株における APC-catenin 複合体，E-cadherin-catenin複合体の発現について検討した．TFF3 (intestinal trefoil factor) は消化管上皮細胞に広く発現を認める trefoil peptide family のひとつであり，腸上皮細胞の遊走を促進し，粘膜傷害の上皮再生を増強するのが考えられている．本研究では，引き続き各種胃癌細胞における APC-catenin 複合体と E-cadherin-catenin 複合体に対する TFF3 の影響を検討する予定である．

4．研究指導者の意見

多くの研究者と協力的かつ精力的に研究を進めており，来年度もその発展が期待出来ます．

研究指導者氏名　服部　隆則　㊞

5．研究報告

別紙形式を参考に、報告本文4000字以上で報告して下さい（枚数自由・ワープロ使用）

タイトル・要旨等は日本語で、KEY WORDS以下は日本語或いは英語で記入して下さい。

研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。

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研究報告書

研究テーマ

胃癌細胞株におけるAPC-catenin複合体，E-cadherin-catenin複合体の発現について

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要旨

目的：E-cadherin-catenin複合体は，Caイオン依存性の細胞間接着の担い手であり，その機能や発現が細胞の運動性や浸潤性と深く関連していることが示されてきた．また最近，β-catenin はE-cadherin だけでなく，epidermal growth factor receptor (EGFR) や adenomatous polyposis coli (APC)蛋白とも複合体を形成していることが明らかとなった．この研究では各種胃癌培養細胞株を用いてAPC-catenin複合体，E-cadeherin-catenin複合体の発現を定量的に検討した．

方法：人胃癌培養細胞株AGS, MKN45（未分化型由来），HSC39, HSC40A, KATO-III（印鑑細胞癌由来），MKN28 and MKN74（分化型癌由来）を用いた．細胞抽出液をSDS-polyacrylamide gel で分画化し，nitrocellulose membraneに転写した後，関心蛋白をモノクローナル抗体とHRPラベルした二次抗体で反応させ，ECL化学蛍光法で検出した．

結果：AGS, KATO-III, MKN45細胞では，正常のE-cadherinは検出されなかった．β-cateninに関してはその変異が知られているHSC39とHSC40Aにおいて約20kDaの欠失が認められた．またp120の発現はMKN28とMKN74以外で減少していた．これらの細胞株にはAPC蛋白の変異はなかったが，HSC39とHSC40Aではその発現が減少していた．

結論：高分化型管状腺癌由来の細胞株では，catenin 複合体の構成要素はすべて完全に保たれているの対して，他の細胞株ではこれらの構成要素に何らかの変異や発現の減少を認めた．Catenin 複合体の発現の異常は，胃癌細胞株の分化様式と緊密に関係していることが示された．

KEY WORDS: 胃癌，細胞接着，APC-catenin 複合体，E-cadherin-catenin複合体

Expression of the APC-catenin and the E-cadherin-catenin complexes in gastric carcinoma cell lines

Purpose: E-cadherin is a calcium-dependent cell-cell adhesion molecule, which forms the key functional component of adherence junctions of all epithelial cells. It interacts with E-cadherin molecules on adjacent epithelial cells forming an adhesive structure, which has been likened to an intercellular zipper (Shapiro, 1995). It plays a key role in the establishment and maintenance of intercellular adhesion, cell polarity and tissue architecture (Takeichi, 1991). Translocation of intercellular contact signals into cellular organization is thought to be mediated by the catenins, which are key regulator molecules in this mechanism. α - catnin (102kDa), β -catenin (94kDa) and γ -catenin (82kDa), are membrane undercoat proteins which, through a series of interactions, link the cytoplasmic carboxy-terminal tail of E-cadherin to the actin cytoskeleton (Nagafuchi, 1989).

Phosphorylation of β -catenin, possibly through interaction with epidermal growth factor receptor (EGFR) and proto-oncogene c-erbB2, is thought to induce a disassembly of the E-cadherin-catenin complex from the actin filament network thus disruption of cell adhesion (Shibamoto, 1994). The Adenomatous Polyposis Coli (APC) gene has been shown to be important in regulating cytoplasmic β -catenin levels. p120 is the most recently discovered member of the catenin family. It is phosphorylated by pp60, a tyrosine kinase known to be associated with the adherens junction (Volberg, 1992), and which induces disruption of adherens junctions and epithelial cell transformation when overexpressed.

To elucidate the role of the E-cadherin-catenin complex in gastric carcinoma, we examined expression and function of complex components in a panel of gastric carcinoma cell lines.

Materials and Methods

Cell lines

Human gastric cancer cell lines, AGS, MKN45, HSC39, HSC40A, Kato-III, MKN28, and

MKN74 were used in this study. Kato-III is a cell line derived from a metastatic pleural effusion secondary to signet ring cell carcinoma of the stomach. AGS and MKN45 are derived from a moderate to poorly differentiated intestinal type gastric carcinoma, while MKN28 and MKN74 are derived from a well-differentiated, intestinal-type gastric carcinoma. HSC39 was derived from malignant ascites arising secondary to a signet ring cell carcinoma, while HSC40A was established from the same original tumor after xenotransplantation of ascitic cells into an athymic BALB/c nude mouse.

Growth and maintenance of cells

Cell lines were maintained in DEME supplement with 10% fetal bovine serum. The cells were cultured at 37℃ in a humidified atmosphere containing 10% carbon dioxide. Cells were tested for mycoplasma at 3-monthly intervals.

Antibodies

Mouse monoclonal IgG antibodies to α -, β - and γ -catenin and p120, were purchased from Transduction Laboratories, Lexington, KY. E-cadherin (HECD-1) was kindly provided by M. Takeichi (Kyoto University).The APC antibody IgG (ALI-12-28) was purchased from AbCam Cambridge UK.

SDS-PAGE and Western blotting

Cell extracts were prepared using Laemmli sample buffer (715mM2- β -mercaptoethanol, 10% glycerol, 2% SDS, 40mM Tris pH 6.8, 1mM EDTA; Laemmli, 1970), with a protease inhibitor cocktail (aprotinin 0.1-2 μ g ml^{-1}, leupeptin 0.5-2 μ g ml^{-1}, PMSF 20-100 μ g ml^{-1}, trypsin-chymotrypsin inhibitor 10 μg ml^{-1}, TPCK 100 μg ml^{-1}(all from Sigma-Aldrich Company Ltd, Poole, UK). Sample protein concentration was determined by Biorad protein assay (Biorad, Hemel Hempstead, UK), using spectrophotometry. SDS-PAGE was performed under reducing conditions using an 8% acrylamide resolving gel, pH 8.8, overlaid with a 4.5% stacking gel pH

6.8. The separated proteins were transferred to nitrocellulose (Millipore, UK), using BioRad apparatus. Visualization of protein bands was achieved by incubation in primary antibody overnight followed by extensive washing, and then in horseradish peroxidase-labelled, secondary specise-specific antibody (Dako Ltd, High Wycombe, UK). The membrane was developed in ECL chemiluminescent reagent (Amersham Life Science, Slough, UK) and exposed to X-ray film.

Expression of E-cadherin and the catenins in gastric carcinoma cell lines

Aberrant expression of E-cadherin and/or catenins was demonstrated in a number of the cell lines examined. Western blot analysis revealed very low levels of E-cadherin in AGS, which were often barely discernible in repeat experiments. Aberrant E-cadherin expression was detected in Kato-III and MKN45. Two protein bands reactive for E-cadherin were detected in Kato 3, one at 120KDa, and a second at a 130 kDa. MKN45 demonstrated one band at the expected molecular weight of 120 KDa and the second at 80 kDa, which is likely to represent the extracellular tryptic product of E-cadherin as previously described (Frixen et al, 1991). E-cadherin was detected at the expected molecular weight of 120 kDa in HSC39, HSC40A, MKN28 and MKN74, although levels of E-cadherin expression were lower in MKN28 and MKN74 compared to control (HT29) and other gastric carcinoma cell lines.

α-catenin expression was absent in AGS, but expressed at the expected molecular weight of 102 kDa in the remaining five cell lines, although levels were reduced in three of these cell lines, Kato-III, HSC39 and HSC40A, compared to control. Beta- catenin was truncated at 80 kDa in HSC39 and HSC40A, as previously described by the originators (Kawanishi et al, 1995). Finally, all seven cell lines expressed γ-catenin and $p120^{cm}$ in comparable amounts and at the expected molecular weight. A band at 300 kDa immunoreactive for APC was detected in all but one cell line, Kato-III, which was negative for APC, suggesting the probable presence of a mutation of the APC gene. A truncated APC protein migrating at 200 kDa was detected in HT29, confirming a previously described mutation.

DISCUSSION

Loss of cadherin-mediated intercellular adhesion is an important contributory mechanism in tumor pathogenesis. It is postulated to remove contact inhibition of proliferation, thus allowing escape from growth control signals (St Croix, 1998). Loss of cadherin-mediated adhesion may also act by potentiating tumor cell detachment from the primary site, and resulting in dissemination of malignant cells to form metastasis at distant sites, and (Birchmeier, 1993). E-cadherin is thus postulated to act as a growth suppressor and invasion suppressor. An intact cadherin-catenin complex is required, however, for maintenance of normal intercellular adhesion, with mutations of either E-cadherin or α/β - catenins being sufficient to disrupt adherens junction function.

In this study, examination of cell morphology, protein expression and molecular organization of the cadherin-catenin complex in seven gastric carcinoma cell lines have demonstrated evidence of widespread dysfunction of calcium-dependent intercellular adhesion mechanisms. E-cadherin is the principal mediator of intercellular adhesion, and adherens junction integrity in epithelial cells.

In conclusion, we have demonstrated frequent abnormalities of expression of the cadherin-catenin complex, correlated with evidence of impaired protein-protein interactions within the cadherin-catenin complex. In the cell lines derived from well differentiated tubular adenocarcinoma, all components of the catenin complexes were completely preserved, whereas some mutation or down regulation of these components were found in the other cell lines. Abnormal expression of the catenin complexes was closely related to the loss of epithelial differentiation in gastric cancer cell lines.

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