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論文内容の要旨

In the multicellular organism, cells in direct contact with neighbouring cells are often linked to each other at specialized intercellular junctions. Intercellular junctions are architecturally differentiated areas of plasma membrane and classified into four types: tight junctions, adherens junctions, desmosomes, and gap junctions. Among them, adherens junctions are the sites of cell contacts which are thought to play crucial roles in the morphogenesis of organs and also in carcinogenesis. In the adherens junctions, Ca^{2+}-dependent cell adhesion molecules (cadherins) are known to work as adhesion molecules and to be bound actin filaments through the well-developed undercoat. Recently, the molecular architecture of the undercoat of adherens junctions has attracted increasing interest, because the undercoat-constitutive proteins are thought to play major roles in the signal transduction and the regulation of cadherin-based cell adhesion. To analyze the molecular architecture of adherens junctions, the isolated adherens junction from rat liver gives powerful cues. When the isolated adherens junction fraction is dialysed against a low-salt alkaline solution, most of the undercoat-constitutive proteins are effectively extracted, leaving the membrane fraction (AJ membrane). The AJ extract is mainly composed of 10 polypeptides including tenuin (400kD), α,β-spectrin (240/235kD), vinculin (130kD), α–actinin (100kD), α-catenin, radixin (82kD), and actin (43kD). Among these constituents, α-catenin is rather resistant to the low-salt alkaline treatment, so that it is mostly left on the AJ membrane. Considering that α-catenin is reported to be tightly bound to the cytoplasmic domain of cadherin molecules, it is tempting to speculate that the undercoat-constitutive proteins which are resistant to the low-salt extraction include some key proteins important for the regulation of cadherin functions. I have attempted to analyze these low-salt-extraction-resistant undercoat-constitutive proteins in detail. For this purpose, I have raised many monoclonal antibodies in mouse using the AJ membrane as antigen. In the first part of this study, my attention was focused on the monoclonal antibodies specific for the 220kD undercoat-constitutive protein which is one of the major constituents of the AJ membrane fraction. Immunofluorescence microscopy with these mAbs showed that this 220kD protein was highly concentrated at the undercoat of adheres junction in various types of tissues. And immunoelectron microscopy with ultrathin cryosection of cardiac muscle cells showed that this protein was located in the immediate vicinity of the plasma membrane in the undercoat of adherens junction. In the cells lacking typical cell-to-cell adherens junction, such as fibroblasts, the 220kD protein was immunofluorescently shown to be concentrated with cadherin molecules at cell-cell adhesion sites. These localization analysis appeared to indicate the possible direct of indirect association of the 220kD protein with cadherin molecules. Furthermore, it was revealed that the 220kD protein and α-spectrin were coimmunoprecipitated with the above mAbs in both the isolated adherens junction and brain. The affinity-purified 220kD protein molecule looked like a spherical particle, and its binding site on the spectrin molecule was shown to be in the position about 10-20nm from the midpoint of spectrin tetramer by low-angle rotary-shadowing electron microscopy. Taking all these results together with biochemical and immunological comparison, I am persuaded to speculate that the 220kD protein is a novel member of the ankyrin family. However, the possibility can not be excluded that the 220kD protein is an isoform of β-spectrin.

These results led me to speculate that this 220kD protein plays an important role in the association of cadherin molecules with spectrin-based membrane skeleton at the cadherin-based cell-cell adhesion sites. In the second part of this study, to clarify the structure and function of the 220kD protein in detail, I cloned its full-length cDNA and sequenced it. Unexpectedly, a similarity and homology search revealed that 220kD protein is quite different from ankyrin or β-spectrin, instead, almost identical to ZO-1, only a part of which had been already sequenced. This identity was confirmed by immunoblotting with anti-ZO-1 antibody against fusion protein made from partial cDNA of the 220kD protein. ZO-1was originally identified as a component exclusively underlying the tight junctions in epithelial cells where cadherins are not believed to be localized. To clarify this discrepancy, I analysed the distribution of cadherins and 220kD proteins by ultrathin cryosection microscopy. I found that, in non-epithelial cells lacking tight junctions, cadherins and 220kD proteins are precisely colocalized at the electron microscopic level, while in epithelial cells bearing well-developed tight junctions, cadherins and 220kD proteins are clearly segregated into adherens and tight junctions, respectively. Interestingly, in epithelial cells such as liver cells where tight junctions are not so well developed, 220kD proteins are detected not only in the tight junction zone but also in the adherens junction zone. Furthermore, transfecting mouse L cells with cDNA encoding N-, P-, E-cadherins demonstrated that cadherins directly or indirectly interact with 220kD protein to colocalize. I discussed the possible function of the 220kD protein with special reference to the molecular mechanism for adherens and tight junction formation.

論文の審査結果の要旨

伊藤雅彦君は、細胞間の接着分子カドヘリンの機能を制御する細胞膜裏打ち蛋白質の同定を目指して本研究を行った。ラット肝臓よりカドヘリンが機能する場である細胞間接着装置Adherens Junction (AJ) を単離し、その膜分画を調製し、それをマウスに注射することによりモノクローナル抗体のライブラリーを作製した。そのなかから分子量220kdの蛋白質を認識するモノクローナル抗体を2クローン選び、その抗原について解析した。この220kd蛋白質は、種々の細胞のAJの細胞膜裏打ち構造に濃縮しており、カドヘリン分子を細胞骨格に結合する上で重要な役割を果たすものと思われた。この蛋白質の構造と機能をさらに解析するために、そのcDNAを単離することを次に試みた。得られたcDNAの構造から、意外なことに、この蛋白質がこれまで上皮細胞でTight Junction (TJ) にのみ局在していると報告されていたZO-1と呼ばれる蛋白質と同一であることが判明した。そこで電子顕微鏡レベルで詳しく220kd蛋白質とカドヘリンの分布を調べたところ、非上皮細胞である線維芽細胞や心筋細胞ではこれら2つの蛋白質は細胞間接着部位に混ざり合いながら局在するのに対し、上皮細胞では前者はTJに、後者はAJにと分離することが分かった。以上の観察はTJとAJの形成機構を考える上でも興味深い。TJとAJの密接な関係は、これまで形態学的知見からさかんに論じられてきたが、本研究によりはじめて分子レベルでの手がかりが得られたことになる。

本論文の示すこの結果は、現在の細胞生物学の中心的課題の一つである細胞接着の制御機構に関する我々の理解を一歩進めるものであり、充分なオリジナリテイが認められた。用いられている技術も多彩であり、データにも充分な説得力があった。論文中の論理の展開も問題がなく、英文の記載も的確であると思われた。また本研究の将来の発展性も充分にあると感じられた。