The structure of desmosomes and their role in malignant disease

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Desmosomes are adhesive intercellular junctions of epithelial cells that link the intermediate filament cytoskeletons of adjacent cells. In epidermis, they consist of eight major constituents, which we shall designate dp (desmosomal proteins) and dg (desmosomal glycoproteins) (Miller et al., 1987). In descending order of relative molecular mass (M), the proteins are dp1 (250,000), dp2 (23,000), dp3 (83,000) and dp4 (75,000), and the glycoproteins dg1 (175,000-164,000), dg2 (130,000), dg3 (115,000) and dg4 (22,000) (Skerrow & Matoltsy, 1974; Gorbsky & Steinberg, 1981; Franke et al., 1982; Cowin & Garrod, 1983; Garrod, 1985, 1986b; Miller et al., 1987). In addition to these are desmocalmin, a high molecular mass calmodulin- and cytokeratin-binding protein (Tsukita et al., 1985), and a glycoprotein of 140,000 M (Jones et al., 1986). Desmosomal proteins and 2 are biochemically and immunologically related to each other, as are dg2 and dg3, while the remaining molecules are distinct (Cohen et al., 1983; Mueller & Franke, 1983; Kapprell et al., 1985).

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Localization of the protein and glycoprotein components within the desmosome

Miller et al. (1987) have used specific antibodies and the high-resolution technique of immunogold labelling of ultrathin frozen sections to record the detailed ultrastructural organization of the major protein and glycoprotein components within desmosomes of bovine nasal epithelium. The molecular distributions found will be described, starting at the intercellular space and progressing through to the cytoplasmic region between the desmosomal plaque and the tonofilaments. (For quantitative data on the distribution of colloidal gold particles in desmosomal profiles, see Miller et al., 1987.) Labelling for dg2 and 3 was shown to be principally located in the 30 nm wide intercellular space, a distribution consistent with the postulated role of these molecules in desmosomal adhesion (Cowin et al. 1984; Garrod 1985, 1986a, b; Garrod & Cowin, 1986). Although labelling was predominantly in the intercellular space, it was unclear whether or not dg2 and 3 are transmembrane proteins since the antibodies may not react with the entire molecule. Labelling for dg1 is present both in the extracellular space and in the cytoplasmic plaque. Desmosomal glycoprotein 1 is therefore a transmembrane glycoprotein whose cytoplasmic portion makes a major contribution to the structure of the desmosomal plaque. (In frozen sections the plaque appears as a 17 nm thick structure with, on its inner cytoplasmic face, a lamina showing a transverse periodicity of 2.6 nm.) Protein 3 is situated within the desmosomal plaque with much labelling close to the cytoplasmic face of the plasma membrane (similar to that found by Gorbsky et al., 1985). Labelling with an antibody recognizing both dp3 and 4 was similar in distribution, but also extended beyond the plaque into the cytoplasm. Desmosomal protein 4 may therefore be positioned close to dp3, but distal to it from the plasma membrane, though this evidence cannot be regarded as conclusive since a dp4-specific antibody was not available.

Desmosomal proteins 1 and 2 were shown to be located predominantly in a region 34 nm wide between the desmosomal plaque and the tonofilaments. These proteins may therefore be involved in joining the plaque to the tonofilaments, rather than being major plaque constituents as previously suggested by Franke et al. (1982).

These results are broadly similar to those obtained by Steinberg et al. (1986) using post-embedding labelling of ultrathin sections. An important difference between the results of the two groups lies in the proposed distribution of the major glycoproteins. The data of Steinberg et al. (1986) suggested that distributions of dg1, 2 and 3 are identical; all three extend from the intercellular space to the inner cytoplasmic face of the plaque. In contrast, our results suggest that dg1 does indeed have this distribution, but that the cytoplasmic extent of dg2 and 3 may be considerably shorter than that of dg1.

To try to clarify the disposition of dg2 and dg3 with respect to the plasma membrane, a series of trypsinization and phosphorylation studies have been carried out with cultured Madin–Darby Canine Kidney (MDCK) cells. A monoclonal antibody, 52-3D, has been shown to react with a conserved epitope on dg2 and 3, since it gave fluorescent staining of many bovine, rat and human epithelia. This epitope must be located either within the plasma membrane or on its cytoplasmic side, since 52-3D does not react with the surface of living cultured MDCK cells, but gives peripheral staining after the cell membranes have been made permeable by acetone fixation. Trypsinization of MDCK cells in the absence of Ca2+ (0.25% (w/v) trypsin with 1 mM EGTA at 37°C for 60 min — no significant decrease in cell viability), followed by Western blotting with 52-3D, yields two polypeptides of M, 28 000 and 24 000. The two undigested proteins have molecular mass of 130 000 and 115 000. Since a monoclonal antibody recognizes one distinct epitope, it is probable that the two fragments come one from each of dg2 and 3.

Incubation of MDCK cells with 32P for 24 h, followed by immunoprecipitation with a polyclonal anti-dg2 and 3 antibody, reveals that only dg2 is phosphorylated under these conditions. Trypsinization of similarly treated cells leads to immunoprecipitation of a single phosphorylated fragment of M, 28 000, thus strengthening the view that the M, 28 000 and 24 000 tryptic fragments are derived, respectively, from dg2 and dg3.

We suggest that these fragments represent the membrane-associated portions of the dg2 and dg3 molecules. The sizes of the fragments appear to define maxima for the sizes of the intramembrane and cytoplasmic domains of the two molecules. The phosphorylation of dg2 confirms that it almost certainly has a cytoplasmic domain. Furthermore, two criteria: size and phosphorylation, demonstrate heterogeneity between dg2 and dg3 in their membrane-associated domains.

Desmosomes and cancer

Reduced strength of adhesion between tumour cells may contribute to the invasive nature of malignancies and could arise through loss of adhesive junctions. There have been varying reports on the numbers of desmosomes in tumour cells. More recently, Steinberg et al. (1986) using post-embedding labelling electron microscopic studies. However, Alroy et al. (1981), in a quantitative morphometric analysis, showed a correlation between reduced numbers of desmosomes and the invasiveness of carcinomas in the urinary bladder. In contrast, Pauli et al. (1978) found no such correlation in chemically induced bladder carcinoma in rats, provided one considered the percentage of cell surface area occupied by junctions.

The advent of anti-desmosomal antibodies has enabled us to examine large numbers of malignant tumours for desmosomes. A survey of 244 malignant carcinomas (breast, 84; bowel, 53; lung, 53; bladder, 40; intracranial metastatic carcinomas, 14) revealed that all of these tumours, regardless of histological grade or degree of differentiation, or whether they were primary or secondary, stained for desmosomal antigens. We conclude that all carcinomas that we have examined possessed desmosomes and that metastatic cell populations do not represent a special group of cells that have lost their ability to form desmosomal junctions.

We have examined the distribution of desmosomal staining in carcinoma of the bowel in more detail. We found that the cells of well- and moderately differentiated tumours showed an organized and polarized distribution of desmosomal staining, similar to that found in normal bowel mucosa. The punctate staining was present only at the lateral contacts between the cells, and concentrated in the terminal bar region near the cell apices. Rather remarkably, the same polarized distribution of staining was found in liver metastases derived from these tumours. This leads to the unavoidable conclusion that metastatic cells derived from moderately well-differentiated carcinoma of the bowel not only retains desmosomes, but must also retain the ability to form desmosomes and to arrange them in a cellular distribution resembling the primary carcinoma.

Since the absence of desmosomes does not explain the metastatic spread of carcinomas, could it be that the junctions of tumours are less stable (i.e. more readily disrupted) than those of normal tissues? Borysenko & Revel (1973) speculated that differences in the sensitivity of desmosomes in different tissues to disruption by EDTA, trypsin or sodium deoxycholate might reflect differences in physiological stability. Watt et al. (1984) and Mattey & Garrod (1986) have shown that desmosomes of cells in tissue culture
exhibit differences in stability to treatment with EDTA or simple reduction of extracellular Ca\(^{2+}\) concentration (< 0.05 mM). These differences vary between cell types and with tissue in the same cell type. Cell lines derived from human bowel carcinomas exhibit similar Ca\(^{2+}\)-dependent stability properties. This prompted us to study stability in neoplastic and uninvolved bowel epithelium in response to extracellular Ca\(^{2+}\) reduction, assessing the results by electron microscopy. When desmosomal junctions of normal bowel were subjected to disruption by removing extracellular calcium, the desmosomes remained intact for up to 2 h, although the non-junctional cell membranes separated and the epithelium became detached from the submucosa. In tumours, many intercellular contacts were readily disrupted by either 4 mM-EDTA or low Ca\(^{2+}\) concentration within 30 min. However, this effect was not specific to desmosomes, but rather involved complete disruption of intercellular contacts.

Treatment of tumours with EDTA or a low concentration of Ca\(^{2+}\) induced much cell degeneration and this may have been primarily responsible for the loss of intercellular contact. Significantly, however, there were groups of cells within the degenerating regions, that appeared to be viable, since their cytoplasm showed a normal density of staining and no vacuolation, while their mitochondria were also unswollen. Furthermore, where contact between such cells and the surrounding degenerate ones was broken, the viable cells were found to be internalizing the half-desmosomes left unpaired by the loss of contact. (Desmosome internalization is a well-documented response of cells to loss of contact (Overton, 1968; Kartenbeck et al., 1982; Mattey & Garrod, 1986).) Within these viable cell groups, desmosomes remained intact although non-junctional membranes were separated in response to Ca\(^{2+}\) reduction.

We feel that these observations may have significance in relation to the mechanism of generation of metastases. Ca\(^{2+}\) depletion may accelerate a disruption process that occurs in tumours, and may illustrate how loss of intercellular contact could release viable cell clumps that may be carried to other locations.

The absolute regularity of occurrence of desmosomes in carcinomas means that anti-desmosomal antibodies should provide reliable reagents for diagnosis of carcinomas. With this in view we have developed two monoclonal antibodies that are proving extremely reliable: 32-2B is an antibody against dpl that reacts with carcinomas in paraffin sections as employed in routine diagnostic pathology (Vilela et al., 1987); while 11-5F is an antibody against dp1 and 2 that react in frozen sections and should prove useful for intra-operative diagnosis of intracranial tumours, meningiomas and metastatic carcinomas (E. P. Parrish, P. Steart, D. R. Garrod, & R. O. Weiler, unpublished work). So far, these reagents appear to be more reliable than existing epithelial markers.

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Structure and evolution of a non-erythroid spectrin, human z-fodrin

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In the vertebrate erythrocyte, a proteinaceous lattice composed of several proteins underlies the cell membrane. This cytoskeletal network is thought to stabilize the cell membrane and control lateral mobility of certain integral membrane proteins which interact with components of the membrane skeleton. The key proteins of the membrane skeleton and their interactions with one another, and with integral membrane proteins, have been extensively characterized by a number of groups (for reviews see Marchesi, 1983; Bennett, 1985). The predominant protein, spectrin, is a dimer composed of 240 kDa α- and 220 kDa β-subunits, which together form a 100-nm-long, rod-shaped molecule. One end of the dimer interacts with other spectrin dimers to form tetramers and possible higher order oligomers, while the other end binds to short F-actin oligomers in a process mediated by the protein 4.1. The actin–spectrin interaction is the best understood. Thus, in the erythrocyte, spectrin plays a vital role linking the actin-head cytoskeleton to the cell membrane.

The demonstration of the existence of non-erythroid homologues of these erythroid proteins (for a review see Moon & McMahon, 1987) has led to the suggestion that most, if not all, non-erythroid cells have a similar membrane skeleton. Such a network, aside from stabilizing the cell