

Growth Control in Mammalian Cells by Cell-Cell Contacts

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Growth of normal diploid mammalian cells *in vitro* is strongly regulated by the actual cell density. Cell-cell contacts via specific plasma membrane glycoproteins whose glycan moieties interact with specific receptors has been found to be a main growth regulatory principle. Malignant growth is suggested to result from impaired function of these receptors.

Density-Dependent Regulation of Growth

The growth of nontransformed diploid mammalian cells *in vitro* is characterized by a cell-density-dependent regulation mechanism: with increasing cell densities, growth is reduced, leading to a stop of proliferation at saturation density. Several hypotheses have been proposed to explain this density-dependent inhibition of growth, such as the release of inhibitory compounds by the cells themselves (1-7), the influence of cell shape on growth (8), the emergence of diffusion boundary layers in confluent cultures, and the exhaustion of nutrients or growth factors of the culture medium (9). In addition, specific cell-cell contacts via plasma membrane proteins have been shown to be of major importance in the growth regulation of normal cells (10-17).

Contact-Dependent Inhibition of Growth

The regulation of growth by specific cell-cell contacts, which is referred to as contact-dependent inhibition of growth, has been studied by the addition of glutaraldehyde-fixed cells or isolated plasma membranes to sparsely seeded cells (18-20). This approach led to a concentration-dependent inhibition of proliferation rate. While in most of the cases only rudimentary data about the molecular properties of the inhibitory compounds have been provided, some groups have shown the glycan

moieties of the plasma membrane glycoproteins to be the active part (10-12,17). Interestingly, in 3T3 fibroblasts, a switch from inactive molecules in sparse cells to inhibitory compounds in confluent cultures has been described (11). This switch resulted from the presence of terminal *N*-acetylglucosamine residues on protein-bound glycans in the case of sparse cells, while the plasma membrane proteins from confluent cultures were free of terminal *N*-acetylglucosamine. A similar phenomenon has been described in human fibroblasts (21); here, the inactivity of plasma membrane glycoproteins from sparse cells was due to the masking of terminal galactose residues by sialic acid. Terminal galactose residues have been shown to be essential for the contact-dependent inhibition of growth of human fibroblasts (22). Another report described the inhibition of growth by glycopeptides obtained after pronase treatment of isolated plasma membranes (12). We were able to show that this is also true for human fibroblasts (20), although an inhibition of growth was found only if the glycopeptides were added in immobilized form.

Characterization of Contactibin

Immobilization on to derivatized silica beads (23) was a *conditio-sine qua non* for an effective inhibition of growth by contactibin, the plasma membrane glycoprotein isolated from human fibroblasts that is responsible for the contact-dependent inhibition of growth (manuscript submitted). Although in immobilized form the glycoprotein was active at picomolar concentrations (10-50 ng/mL), even at concentrations of up to 1 µg/mL, the glycoprotein had only marginal growth inhibitory activity when added in soluble form. The immobilization may have several effects that are responsible for the observed inhibition of growth. On one hand, this

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method ensures that only contact-dependent phenomena are induced. On the other hand, the immobilization obviously leads to local concentrations of contactibin, which are necessary for a biological effect. It is well known from cell adhesion studies, for example, to immobilized sugars (24), that threshold concentrations exist which provide a none-or-all reaction with respect to adhesion. In addition, it has been shown that multimerization of univalent, inactive ligands leads to the generation of highly active compounds.

Contactibin has been found to be a 60 to 70 kDa glycoprotein with 20 to 30% glycans, as judged from the running behavior in SDS-PAGE. Based on immunological studies with anti-contactibin antibodies, contactibin is located in a detergent-extractable form in the plasma membrane, although to a certain extent it is anchored to cytoskeletal elements. In this form, contactibin is resistant to detergent extraction. The conditions that regulate the expression of contactibin in a free and bound form are not known, but cytoskeletal binding is suggested to have important functions for the biological activity on the basis of the observations with the proliferation test system.

The addition of anti-contactibin antibodies to confluent cultures of human normal fibroblasts resulted in a release from contact-dependent inhibition of growth. Treated cultures showed a 2- to 3-fold higher saturation density and the appearance of focal growth as well as criss-cross growth pattern. Removing contactibin from detergent extracts of isolated plasma membranes by matrix-bound anti-contactibin antibodies resulted in a complete loss of the inhibitory activity of these extracts, indicating that contactibin is the only glycoprotein involved in the contact-dependent inhibition of growth.

It has been found that confluent cultures of human fibroblasts can be stimulated to go through a round of cell division by the addition of high serum concentrations or of certain growth factor combinations (EGF, FGF, and transferrin) or of urea (0.35 M) (unpublished observations). In all cases a release of contactibin into the culture medium has been observed. This may suggest a causal connection and give an indication of the necessity of the (proteolytic?) release of contactibin to allow proliferation in confluent cultures.

Transformation has been shown to be connected with a loss of responsiveness toward cell-cell contacts with respect to growth control. This loss is not due to changes of contactibin, as transformed cells have been found to synthesize contactibin in biologically active form. This is true for chemically transformed C3H 10T1/2 cells and for human fibrosarcoma cells (19). On the other hand, it has been found that human tumors (sarcomas as well as carcinomas) express various forms of contactibin. Although an organ-specific pattern of contactibin variants has been found, tumors express contactibin-forms that are not organ restricted. The variants seem to arise from altered glycosylation of contactibin.

By development of an aggregation assay, we were able to show that contactibin binds specifically to proteins of a detergent extract of human diploid fibroblasts.

This binding was inhibited by the presence of anti-contactibin antibodies or of plasma membrane proteins. Only marginal binding of contactibin has been observed to plasma membrane proteins of SV-40 transformed human fibroblasts, indicating that in transformed cells the postulated receptor is defective or is no longer synthesized. In addition to our findings, Peterson and Lerch have shown that contact-dependent inhibition of growth can be restored in transformed cells by fusing plasma membranes of nontransformed cells with their transformed counterparts (25), giving evidence that the primary growth control defect in transformed cells resides within the plasma membrane. The actual data do not allow a clear answer to the question of whether the receptor in transformed cells is defective or is no longer synthesized, but the results of several studies point to a defective state. It has been shown that normal cells can be phenotypically transformed by inhibitors of phosphotyrosine phosphatases (26). Furthermore, inhibitors of cyclic phosphodiesterase lead to the appearance of contact-dependent inhibition of growth in transformed cells (27).

Conclusions

Sparsely seeded cells express contactibin in a higher sialylated form than confluent cells. This could be a reflection of a cell-cycle specific synthesis of contactibin, or could be due to a release of terminal sialic acids by a membrane-bound sialidase upon establishment of cell-cell contacts.

Upon unmasking of terminal, β -glycosidically linked galactose residues, contactibin can bind to a specific receptor. The receptor is suggested to be highly specific for the sugar moieties of contactibin, as only the glycans have biological activity and as other glycoproteins with terminal β -glycosidically linked galactose residues (e.g., asialofetuin, alpha acid glycoprotein) have no effect on growth.

Once a closed monolayer has developed, reinitiation of proliferation is postulated to be possible only when the cell-cell contacts formed by contactibin and the contactibin receptor are broken. This may be due to the action of proteases, which are thought to be induced by growth factors or by urea. The growth inhibitory signal starting from the contactibin receptor is thought to be triggered by phosphorylation reactions.

Transformation leads to a defective receptor molecule that is no longer able to bind to contactibin. Therefore, growth inhibitory cell interactions are suppressed. The defect may be due to altered phosphorylation or altered and defective synthesis. Activation of certain oncogenes may lead to enhanced phosphorylation, perhaps preferentially of the contactibin receptor, and thus enable cells to escape the contact-dependent inhibition of growth.

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