Variant choriocarcinoma (BeWo) cells that differ in adhesion and migration on fibronectin display conserved patterns of integrin expression

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Summary

Starting from the BeWo choriocarcinoma cell line, two stable variant cell lines (epi and lc) were isolated. Epi cells displayed an epithelioid colony morphology while lc were fibroblastoïd. lc cells attached and spread on fibronectin-coated surfaces at significantly lower density of fibronectin than epi or the parent cell line. lc also migrated more efficiently to fibronectin in a trans-filter assay than either epi or parent cells. Integrin expression by the cell lines was investigated by flow cytometry and immunoprecipitation from surface-labelled cells with a panel of subunit-specific antibodies. Integrins α2β1, α5β1, αvβ1 and αvβ4 were detected in each case, and levels of expression were identical in the two variant lines. Anti-functional antibodies were used to probe the role of integrins in fibronectin- and vitronectin-mediated adhesion. Complete inhibition of adhesion to fibronectin was observed with anti-β1 antibody, and partial inhibition with anti-α5, suggesting that integrin α5β1 is mainly responsible for the interaction. Adhesion to vitronectin was inhibitable using anti-αv and anti-β1 antibodies, suggesting that integrin αvβ1 is active in these cells as a vitronectin receptor. There was a correlation between the altered morphology of the variant cells and alterations in the distribution of integrin αvβ4 and laminin in monolayer cultures. The results support the idea that fibronectin may mediate the migratory behaviour of extravillous trophoblast in vivo. Switch to a more migratory phenotype may be mediated by the selective activation of integrins and altered interaction with basement membrane.

Key words: trophoblast, integrin, fibronectin, BeWo.

Introduction

At implantation the human uterine epithelium is penetrated by trophoblast, which then proceeds to invade and remodel the underlying stroma (Aplin, 1991a; Weitlauf, 1988; Denker, 1990; Lindenberg et al. 1990; Schlafke and Enders, 1975). Meanwhile, hormonally induced differentiation is occurring in the maternal stroma, giving rise to decidua (Glasser et al. 1991). Subsequently, from the 3rd to the 18th week of pregnancy, cytotrophoblast migrates from the developing placenta into the maternal decidual interstitium and spiral arteries, where transformation of the arterial intima occurs from a collagenous-elastic matrix to fibrinoid (Pijnenborg et al. 1980). During these processes interaction occurs between trophoblast and the maternal decidual extracellular matrix, which contains interstitial collagens, fibronectin, and basement membrane components (Aplin et al. 1988; Aplin and Jones, 1989). Indeed, it is likely that the maternal matrix plays a permissive role in the adhesion and migration of trophoblast as it escapes from the tips of anchoring villi into the maternal environment (Aplin and Charlton, 1990). Extravillous cytotrophoblast also contributes to its own matrix environment (Feinberg et al. 1991).

Although it is clear that extravillous trophoblast exhibits certain phenotypic characteristics that distinguish it from villous cytotrophoblast (Enders, 1968; Gosseye and Fox, 1984; Kurman et al. 1984; Loke et al. 1990; Yagel et al. 1988; Aplin, 1991a; Feinberg et al. 1991), it is difficult to isolate this cell population for study of its migratory behaviour in vitro. An alternative strategy is based on the availability of choriocarcinoma cell lines such as BeWo which express certain of the characteristics of normal trophoblast. The BeWo cell line is heterogeneous on several criteria (Aplin, 1991a): presence of a population of (approximately 1%) multinuclear giant cells (Martell and Ruddon, 1990), expression of the placental form of alkaline phosphatase, placental lactogen, choric gonadotrophin, and other trophoblast-specific markers. We sought to isolate from BeWo cells that might exhibit some of the properties of the motile extravillous cell population. We now describe the characteristics of one such lineage, lc, and compare it with another lineage, epi, which exhibits an epithelioid, non-migratory pattern of behaviour. We compare the adhesion of the two
sublines to fibronectin and their patterns of integrin expression.

Materials and Methods

Cell culture
BeWo cells were obtained from the European Animal Cell Culture Collection (Porton Down, UK) and were cultured in an equal mixture of Dulbecco's modified Eagle's medium and Ham's F12 containing 2 mM L-glutamine, 100 i.u./ml penicillin, 10 µg base/ml streptomycin and 10% fetal calf serum.

Establishment of cell lines
BeWo cells grow as colonies with complex and varying morphology. After low-density (approximately 1 cell/cm²) plating and up to 3 weeks' culture, colonies were observed taking the form of smooth-edged islands containing polygonal cells mainly in monolayer, many with obvious intercellular borders. Other colonies were apparent that exhibited ragged edges comprising elongated fibroblastic cells with some tendency to migrate from the periphery of the parent colony. These latter colonies grew densely with cells in the centre piling up on top of one another, and tended to proliferate more rapidly than the epithelioid colonies. Other colony types with mixed morphology were also observed.

Colonies exhibiting fibroblastic morphology were isolated after plating the parent cells at low density and culturing them for several weeks. Sparserly growing colonies were then visible by eye and could be picked, dispersed with trypsin and expanded. These cultures were screened for fibroblastic and epithelioid colonies, and the selection process repeated until all colonies in a culture flask exhibited similar characteristics. A cell line isolated in this way was named lc and is described herein.

Colonies exhibiting epithelioid morphology were slower-growing than their fibroblastic counterparts, and were selected using a different procedure. After seeding at low density and growth to colony sizes of several hundred cells, fibroblastic and smaller epithelioid colonies were removed by scraping. This procedure was repeated until a single large epithelioid colony remained in the flask. This was then dispersed by trypsinisation and the resulting culture was grown up and then subjected to a repeat selection. The cell line to be described was named epi. The two variant phenotypes were stable over a period of 12 months' continuous culture.

Antibodies
Anti-integrin antibodies were as follows: anti-α₁ monoclonal TS2/7 was from M.Hemler; anti-α₂ monoclonal 12F1 from V.Woods; anti-α₃ polyclonal J143 from H. Grahnick; anti-α₄ monoclonal P3E3 from E. Wayner; anti-α₅ monoclonal mAb16 and anti-β₁ monoclonal mAb13 from K. Yamada; anti-α₆ monoclonal BIE5 from C.H.Damsky; anti-α₇ monoclonal GoH3 from A. Sonnenberg; anti-α₈ and anti-β₃ polyclonals from D.Cheresh; anti-β₂ monoclonal TS1/18 from T. Springer; anti-β₃ monoclonal 439-9B from S. Kenne1; anti-β₅ monoclonals 5B5 (Sonnenberg et al. 1991) and G71 (Aplin and Seif, 1985) were raised in this laboratory; polyclonal anti-α₂β₁ was from Telios, La Jolla, USA; polyclonal anti-laminin was from BRL, UK.

Immunofluorescence and flow cytometry
FITC-conjugated second antibodies were obtained from Dako. Rhodamine-phalloidin, which was used to visualise actin, was from Molecular Probes. Cells grown on glass coverslips were washed twice in PBS and fixed in cold acetone before staining as described previously. For flow cytometry, cells were dispersed by trypsinisation and stained in suspension using anti-integrin monoclonal antibodies at 1/10 (α₄α₅β₁α₃) or 1/1000 (β₁α₃), followed after three washing steps by FITC-conjugated mouse or anti-rat immunoglobulin (Dako) at 1/200. After three further washes the cells were dispersed by pipetting and analysed on a Beckton-Dickenson FACScan using Consort 30 software. Controls included secondary antibody alone as well as control first antibodies to integrin subunits absent from trophoblast. These two types of control were indistinguishable from one another. Data are tabulated as percentages of positive cells after gating out background fluorescence at a level determined in the appropriate control. Mean fluorescence is also cited (arbitrary units); this allows comparison of fluorescence between the three cell populations using the same antibody.

Spreading assay
This was essentially as previously described (Aplin and Foden, 1984). Plasma fibronectin (New York Blood Bank) or vitronectin (Sigma) were dissolved in PBS and stored frozen at 50 and 250 µg/ml, respectively. They were further diluted and used to coat tissue-culture grade plastic multiwells overnight. Wells were then washed and incubated in bovine serum albumin (100 µg/ml) for 1 h. Cells were washed and brought into suspension with 0.05% trypsin/0.02% EDTA (Gibco, UK), the trypsin neutralised with an equal volume of 0.05% soybean trypsin inhibitor (Sigma, UK) and the cells resuspended and washed twice in medium without serum. Cells were then added to the washed coated wells.

For assaying the effect of various anti-integrin antibodies on spreading, cells were suspended in antibody at the stated dilution prior to seeding into wells. On the basis of the data shown in Fig. 3, a fibronectin coating concentration of 5 µg/ml was chosen for antibody-mediated inhibition of cell spreading. For vitronectin a similar concentration study was carried out in order to establish an optimal concentration for parent cell spreading; 80% of the maximal response was seen at 5 µg/ml and so this coating concentration was chosen for the antibody inhibition experiments.

Where inhibition of spreading was observed, cell attachment could also be inhibited by further increasing the concentration of antibody. However, cell spreading was the criterion used to monitor the activity (Aplin, 1991b). At least 200 cells were scored in at least 10 fields and the data plotted ±s.e.m. Significance was assessed at each concentration using the Mann-Whitney U-test, and the three cell lines were significantly different from one another except in the highest and lowest parts of the fibronectin concentration range (fully rounded or fully spread cells), where the data converge. Data were also analysed by non-linear regression methods using a portfolio of growth models from the package SIMFIT (details available from W.G.Bardsley, University of Manchester). Models with 3 or 4 parameters were selected and in each case the best-fit values were significantly different in the three cell lines tested.

Form factor analysis
Cells were removed by incubation at 37°C with 0.05% (w/v) trypsin. The trypsin was inactivated with an equal volume of 0.05% (w/v) soybean trypsin inhibitor in PBS. Aggregates were dispersed by gentle pipetting, then cells were counted using a haemocytometer and diluted in complete medium to give a total of 10⁵ cells in a 25 cm² flask. Incubation proceeded at 37°C for 2 h to allow cells to attach and spread. The form factor of at least 50 cells was measured as 4π(area)/(perimeter)² on an image analyser linked to an inverted phase-contrast microscope (Measuremouse, Analytical Measuring Systems, Oxford, UK; Leitz,
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A measure of the longest length across a cell was similarly taken. The data are cited ± s.e.m. and the significance of differences was assessed using the Mann-Whitney U-test.

**Transfilter migration assay**

Cell migration was quantified using a 48-well microchemotaxis chamber (Neuro Probe Inc., USA). Human pooled plasma fibronectin (pFN; New York Blood Bank) was diluted in migration medium (culture medium without serum). A 25 µl sample of each dilution was introduced into triplicate wells. The filled wells were covered with a polyvinylpyrrolidone-free polycarbonate filter (8 µm pore size, Nuclepore Corp., USA) that had been coated with 5 µg/ml of gelatin as described by Postlethwaite et al. (1976). The chamber setup was completed according to the manufacturer’s instructions.

A single cell suspension was obtained as described above. Cells were resuspended in migration medium at 5 x 10^5 cell/ml and 50 µl was introduced to top wells. The assembly was incubated at 37°C. After 5 h the filter was removed and washed in PBS, fixed in methanol (1 min), stained in Harris’s haematoxylin (5 min) (BDH, UK) and “blued” in tap water. The filter was cut into 4 parts and each was mounted in aqueous mountant (BDH, UK).

Cells that had migrated and cells left behind were counted using a x100 objective in 5 fields from each well (i.e. 15 fields per pFN concentration). The cell migration index (CMI) was calculated as:

\[(\text{mean no. cells migrated}) \times 100/(\text{mean no. of cells in monolayer})\]

and cited ± s.e.m.

**Results**

**Characterisation of epi and lc variant sublines**

The lc subline grew as colonies with ragged edges made up of elongated bipolar cells (Fig. 1a,b). In the centre of larger colonies polygonally shaped cells were also visible, and this was more apparent as colonies became larger (Fig. 1d). In the centre of colonies cells tended to pile up (Fig. 1a,b,c).

The epi subline grew as colonies with smooth edges (Fig. 2a) containing polygonal cells (Fig. 2c,d), in some areas of which linear intercellular borders were clearly visible by phase-contrast (Fig. 2d). Less multilayering of cells was apparent, and in larger colonies ‘domes’ sometimes appeared comprising roughly circular areas of cells detached from the substrate (Fig. 2b).

Actin was visualised by staining permeabilised cells with rhodamine-phalloidin (Figs 1c,2c). In flattened polygonal areas of cells, intercellular borders were easily visible as a result of their association with prominent bundles of microfilaments. Such areas of cells were much more frequent in epi than in lc cells. Bright homogeneous actin staining was characteristic of cells that had detached from the substrate and formed an upper layer in the colony. This was much more common in lc than in epi.

Where the cells grew in monolayer, actin staining in combination with examination under phase-contrast optics also

![Fig. 1. Subline lc shows elongated bipolar cells within and at the edges of small colonies (a,b). At densities close to confluence (c,d) a more epithelioid appearance is evident and actin (NBD-phalloidin staining, c) is present at intercellular borders. Brighter fluorescence in c corresponds to areas of bilayering of the cells, the upper layer cells being less flattened. a, ×85; b, ×170; c,d, ×75.](image-url)
enabled a clear distinction to be made between mononuclear and multinuclear cells; this is not possible on the basis of phase-contrast alone because cell borders are not obvious in all areas of either subline or, indeed, the parent cells. All three cell lines exhibited approximately the same low frequency (<1%) of multinuclear cells.

Differences in colony morphology between the two sublines became readily apparent after several days in culture. However, differences in the average shapes of individual cells were also apparent immediately after plating; epi cells spread in the presence of serum to produce a mean perimeter of 174 ± 6 µm, a mean longest length of 61 ± 3 µm, and a mean form factor of 0.67 ± 0.01. lc cells exhibited a mean perimeter of 208 ± 9 µm, a mean longest length of 75 ± 3.5 µm and a mean form factor of 0.51 ± 0.02. The differences are all highly significant (P<0.001) and these data indicate that lc cells are on average more elongated than epi.

Adhesion and migration of sublines to fibronectin

The parent and variant cell lines were tested for the ability to attach and spread on a substratum containing various densities of adsorbed plasma fibronectin. The data, shown in Fig. 3, demonstrate that lc cells spread on fibronectin at a significantly lower density (P<0.001) than epi cells, the latter requiring roughly a two-fold higher concentration of fibronectin. The parent cell line fell between the two vari-

**Fig. 2.** Subline epi demonstrating epithelioid colonies with smooth edges (a) and the occasional formation of raised ‘domes’ in denser areas (b). Actin staining (NBD-phalloidin, c) demonstrates prominent bundling at intercellular borders which, in some areas of cell monolayer, are also evident in phase contrast (d). Actin staining also shows that some cell bilayering occurs (brighter areas in c). a, ×85; b, ×170; c,d, ×370.

**Fig. 3.** Spreading of BeWo parent cells and sublines epi and lc on substrata coated with increasing density of fibronectin. Cells were plated into dishes containing fibronectin carpets and allowed to attach and spread for a period of 90 min. The (logarithmic) horizontal axis shows the concentration of fibronectin used to coat the dish. (+) BeWo; (•) lc; (□) epi. Data points are given ± s.e.m. epi cells require significantly more fibronectin, and lc cells significantly less fibronectin to spread than BeWo.
Differences were also apparent between the variant cell lines in their abilities to migrate across a porous filter up a gradient of fibronectin concentration. Fig. 4 shows that lc cells did not migrate significantly more rapidly than either epi or parent BeWo cells across the filter in the absence of fibronectin. The introduction of fibronectin into the target chamber led to a dose-dependent increase in the migration of all three cell lines. However, the enhancement of migration of lc cells was greater than for epi or parent cells leading to significant differences between lc and the other two cell lines.

**Expression of integrins by BeWo and variant cells**

The observed differences in adhesion and migration of the two variant cell lines led us to test the hypothesis that they might express different repertoires of integrins at the cell surface. First, we characterised integrin expression by means of an immunoprecipitation analysis from surface-iodinated cells with a panel of subunit-specific antibodies. The results, shown in Fig. 5, indicate the presence of integrins $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_1$, and, very prominently, $\alpha_6\beta_4$. The figure shows the results for subline lc, and identical results were obtained using epi cells.

To compare quantitatively levels of expression of integrin subunits in the parent and two variant cell lines, flow cytometry was used. Data are summarised in Table 1. This confirms the presence of integrin subunits $\alpha_5$, $\alpha_6$, $\alpha_v$, $\beta_1$, and $\beta_4$. Subunit $\alpha_2$ was also confirmed as being present (not shown). Subunits $\alpha_1$ and $\alpha_4$ were not detected. No significant differences were observed in the level of expression of $\beta_1$. No $\beta_1$ subunit is precipitated by anti-$\alpha_6$, suggesting the absence of integrin $\alpha_6\beta_1$. Identical results were obtained from epi cells.
of any of the integrin subunits in the two variant cell lines. It is noteworthy however that both variants show differences from the parent line (see $\alpha_5$ and $\beta_4$).

Immunofluorescence was also used to examine the surface distribution of integrin molecules in cell cultures. The $\beta_1$ integrins exhibited a surface fluorescence which appeared both at intercellular contact areas (Fig. 6a) and at the free edges of cells at the periphery of colonies (Fig. 6b). Staining with polyclonal antibody to integrin $\alpha_5\beta_1$ gave similar results (Fig. 6c). There were no obvious differences between the different cell lines. In epi cells, integrin $\alpha_6\beta_4$ was concentrated at intercellular borders, giving rise to regular polygonal areas of cells to a regular linear network of staining (Fig. 7b). This was not apparent in lc cells, which tends to exhibit a more diffuse staining with rounded cells adhering to the upper surface of substratum-attached basal cells appearing more prominently labelled (Fig. 7d). Cells were also stained for fibronectin, which was absent, and laminin, which formed a regular intercellular fibrillar deposit in epi cells (Fig. 7a), apparently running along intercellular borders, where it often codistributed with integrin subunits $\alpha_6$ and $\beta_4$. Laminin was also expressed by lc cells.

Table 1. Flow cytometric evaluation of integrin subunits on BeWo parent cells, epi and lc cells

<table>
<thead>
<tr>
<th>Subunit</th>
<th>epi</th>
<th></th>
<th>lc</th>
<th></th>
<th>BeWo</th>
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<tr>
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<td></td>
<td>0</td>
<td></td>
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<tr>
<td>$\alpha_4$</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
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</tr>
<tr>
<td>$\alpha_5$</td>
<td>94.1</td>
<td>221</td>
<td>94.2</td>
<td>244</td>
<td>90.6</td>
<td>347</td>
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<tr>
<td>$\alpha_6$</td>
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<td>283</td>
<td>96.5</td>
<td>268</td>
<td>95.3</td>
<td>295</td>
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<tr>
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<td>59</td>
<td>55.6</td>
<td>80</td>
<td>31.6</td>
<td>88</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>96.1</td>
<td>277</td>
<td>96.0</td>
<td>268</td>
<td>94.6</td>
<td>342</td>
</tr>
<tr>
<td>$\beta_4$</td>
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<td>91</td>
<td>81.0</td>
<td>95</td>
<td>71.1</td>
<td>93</td>
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%: Percentage of cells expressing fluorescence above background levels; m.f., mean fluorescence in arbitrary units.

Fig. 6. Immunofluorescence of integrin subunit $\beta_1$ within (a) and at the edge (b) of a BeWo cell colony. (c) Shows a similar result obtained using a polyclonal antibody to $\alpha_5\beta_1$. Arrow in (b) indicates a punctate deposit at the outer edge of a cell. Otherwise the major staining is at intercellular borders. ×370.

Fig. 7. Immunofluorescence of epi (a,b) and lc (c,d) sublines with antibodies to laminin (a,c) or integrin $\alpha_6$ (b,d). In epi cells (a), intercellular laminin-containing fibrils are much more prominent than inlc cells (c). Integrin $\alpha_6$ is present mainly in association with epi cell edges in the monolayer (b) while in lc cells it has a more diffuse distribution (d). ×370.
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(Fig. 7c), but the observed spots and short fibrils were not arranged in a way that obviously reflected the organisation of the cell layer. The distribution of integrin subunit $\beta_4$ was similar to that seen for $\alpha_6$ (Fig. 8a,b).

**Effect of anti-integrin antibodies on adhesive behaviour**

Antibodies to integrin subunits with the ability to inhibit aspects of their adhesive function were tested to determine the role of the four detected dimers in adhesion to fibronectin. Monoclonal antibody BIE5 (Fig. 9a) to the $\alpha_5$ subunit had a significant inhibitory effect, leading to a 70% reduction in the spreading of the cells at the highest antibody concentration used. Antibody mAb13 to the $\beta_1$ integrin subunit gave a full inhibitory effect (Fig. 9b). The anti-$\beta_1$ monoclonal antibody also inhibited trans-filter migration of BeWo cells to fibronectin (not shown). No conclusive inhibitory activity was observed in the migration assay with available anti-$\alpha$-chain reagents.

Antibodies to $\alpha_v$ (rabbit immunoglobulin; Fig. 9c) had no inhibitory effect on BeWo cell spreading on fibronectin. BeWo cells were found to attach and spread efficiently on vitronectin-coated surfaces and this could be inhibited by either anti-$\beta_1$ or anti-$\alpha_v$ antibodies (Fig. 10).

Antibodies to the integrin $\alpha_6\beta_4$ complex (GoH3 to $\alpha_6$; 439-9B and 5B5 to $\beta_4$) had no effect on BeWo cell spreading on fibronectin (not shown). The same antibodies were added to monolayer cultures of BeWo cells to test for any possible effect on cell-cell contacts or cell or colony morphology. No effects were detected.

**Discussion**

During placentation, from about the 3rd week of pregnancy,
behaviour is epiboly, elongated bipolar cells also occasionally appear at the edges of colonies, detach and migrate. It clearly differs in several important respects from its villous counterpart (Enders, 1968; Gosseye and Fox, 1984; Kurman et al. 1984). The parent line shows a more homogeneous stable epithelioid phenotype than the parent line. These cells are significantly weaker in their adhesive and migratory response to fibronectin, and may represent a pattern of behaviour closer to that of the villous cytotrophoblast. It should be emphasized, however, that neither variant cell line differentiates with higher efficiency than the parent line into syncytial cells.

Fig. 10. BeWo cell adhesion to vitronectin: the effects of anti-integrin antibodies. Cells were plated onto wells precoated with 5 µg/ml vitronectin. Concentration-dependent inhibition is observed with antibodies to α6 (a) or β1 (b) subunits.

cytotrophoblast undergoes an epithelial-mesenchymal transformation as it loses contact with the villous basement membrane and escapes from the tips of anchoring villi. The resulting population of 'extravillous' trophoblast proceeds to invade and colonise adjacent and deeper maternal tissues (Pijnenborg et al. 1980; Benirschke and Kaufman, 1990; Aplin, 1991a; Aplin et al. 1992). Little is known of the properties and function of this form of trophoblast, though it clearly differs in several important respects from its villous counterpart (Enders, 1968; Gosseye and Fox, 1984; Kurman et al. 1984; Benirschke and Kaufman, 1990; Loke et al. 1990; Aplin, 1991a).

We have used the BeWo choriocarcinoma cell line as a model system for investigating trophoblast interaction with extracellular matrix. In previous studies (Aplin and Charlton, 1990) we demonstrated that BeWo cells are morphologically heterogeneous, containing both epithelial-like and fibroblastic cells in addition to a small proportion of large flattened multinucleate giant cells. Some interconversion between the cell forms occurs. Time-lapse studies of BeWo cells indicated that whereas the dominant form of motile behaviour is epiboly, elongated bipolar cells also occasionally appear at the edges of colonies, detach and migrate away into the surrounding substratum. They do not in general, however, proceed more than a few cell diameters from the colony of origin. Similar patterns of motile behaviour were observed when BeWo cells were cultured on collagen gel substrata; elongated cells appeared at the peripheries of colonies, but generally failed to escape into the interstices of the gel matrix. Culture in the presence of serum on substrata containing fibronectin or laminin gave similar results.

Isolated epithelial cells in culture can take on a range of forms including the polarised elongated shape more characteristic of fibroblasts (Brown and Middleton, 1985). Once in contact with neighbouring cells, however, their shapes are influenced heavily by the formation of intercellular contacts characteristic of epithelia (Mege et al. 1988), and epithelial sheets usually consist mainly of fairly regular polygonal cells. However, there are reports of elongated bipolar cells appearing in pure epithelial cell cultures. Hay and coworkers (Greenburg and Hay, 1982; Zuk et al. 1989) found that corneal epithelial cells could transform into a mesenchyme-like phenotype (elongated bipolar cells with the capacity to migrate independently) in three-dimensional collagen gels. Valles et al. (1990) have reported that cells with mesenchymal characteristics can be produced in a bladder carcinoma cell line by treatment with acidic FGF. In each case elongated shape and fibroblastic migratory characteristics coincided.

In the present studies we have isolated and characterised a subline of fibroblastic cells (lc) arising spontaneously in mass cultures of BeWo cells. lc shows some spontaneous migratory activity in two-dimensional assay systems, and attaches, spreads and migrates more efficiently on fibronectin than the parent line. Fibronectin is present in maternal decidua in locations where trophoblast migration occurs (Aplin et al. 1988; Aplin and Jones, 1989; Earl et al. 1990; Feinberg et al. 1991) and may provide transient anchorage for migrating cells in vivo. Thus an enhanced ability to adhere to and migrate on fibronectin may be consistent with the requirements of an extravillous phenotype. The presence of the lc phenotype within the parent line therefore supports the contention that features of (or leading to) the extravillous lineage are expressed. However, lc cells fail to migrate into collagen gels (unpublished data) and their migratory activity on plastic culture substrata falls short of that observed in cultured fibroblasts. Therefore it is clear that lc cells do not show all the characteristics expected of the fully invasive trophoblast phenotype.

We have also isolated a BeWo cell subline (epi) exhibiting a more homogeneous stable epithelioid phenotype than the parent line. These cells are significantly weaker in their adhesive and migratory response to fibronectin, and may represent a pattern of behaviour closer to that of the villous cytotrophoblast. It should be emphasized, however, that neither variant cell line differentiates with higher efficiency than the parent line into syncytial cells.

Integrin expression in trophoblast in vivo has been characterised by Taylor et al. (1989), Korhonen et al. (1991), Aplin (1992), Aplin et al. (1992), and Damsky et al. (1992), who all report that the α6β4 subunits codistribute in villous cytotrophoblast. Both subunits are absent from interstitial extravillous trophoblast, though they can be detected on cytotrophoblast columns. Thus the expression by BeWo cells of α6β4 is reminiscent of a villous cytotrophoblastic rather than an extravillous phenotype. In contrast to these
data, Hall et al. (1990) have reported that the choriocarcinoma cell line JAr expresses integrin $\alpha_6\beta_1$ and adheres efficiently to laminin. These cells are more uniformly epithelioid in culture, lack $\alpha_6\beta_4$, and also express integrin $\alpha_\beta_1$, which confers on them the ability to adhere to collagen type IV, and the fibronectin receptor $\alpha_\beta_6$. Thus BeWo (which lack $\alpha_\beta_1$ and $\alpha_6\beta_1$) and JAr cells are clearly distinct in terms both of adhesive behaviour and integrin expression. Although it is clear that in some situations integrin $\alpha_6\beta_1$ also acts as a laminin receptor (Lee et al. 1992), the poor adhesion of BeWo cells to EHS laminin suggests that in this cell line, $\alpha_6\beta_1$ is not a receptor for this laminin isoform.

We have demonstrated here that integrin $\alpha_\beta_1$ is at least partially responsible for BeWo interaction with fibronectin; the interaction is inhibited by antibody to either of these subunits. Although the cells express significant amounts of $\alpha_\beta_1$, which can also act as a fibronectin receptor (Vogel et al. 1990), no inhibitory effect on cell spreading was obtained with antibody to $\alpha_\beta_1$. Instead, our data show that BeWo cells adhere efficiently to vitronectin, and that this is inhibitable by antibodies to either $\alpha_\beta_1$ subunits, suggesting that $\alpha_\beta_1$ acts as a vitronectin receptor in these cells. Despite the observed differences in their binding affinities for fibronectin, the BeWo-derived variant sublines are identical in their patterns and levels of integrin expression. There is considerable evidence to suggest that modulation in situ of integrin affinity for an extracellular ligand provides the cell with one mechanism for regulation of its interactions with extracellular matrix (Shimizu et al. 1990; Springer, 1990; Neugebauer and Reichardt, 1991; Hynes, 1992). In differentiating keratinocytes, loss of $\alpha_\beta_1$-mediated adhesion to fibronectin occurs without down-regulation of the integrin at the cell surface (Adams and Watt, 1990). The intracellular signals involved in regulating integrin affinity are at present poorly understood.

It is probable that the alteration from a static mode of adhesion to a motile phenotype in vivo, as in trophoblast differentiation down the extravillous pathway, involves alterations in the pattern of integrin expression as well as activation/deactivation of existing integrins. Thus, for example, integrin $\alpha_6\beta_1$ disappears from the cell surface after escape of the cytotrophoblast from anchoring villi in vivo (Korhonen et al. 1991; Aplin, 1992; Damsky et al. 1992); at the same time, increases are evident in the expression of $\beta_1$ integrins (Korhonen et al. 1991; Aplin et al. 1992; Damsky et al. 1992), which are not abundant on villous trophoblast (Taylor et al. 1989). It is clear that analysis of integrin expression in the villous and extravillous trophoblast populations will give valuable insight into utilisation of extracellular ligands by trophoblast in both villous and extravillous environments. It will also give further insight into the control of differentiation in the trophoblast lineage.

We are grateful to Drs. Zoltan Szekanecz and Anne Ager for assistance with the FACS analysis.

References


(Received 16 March 1992 - Accepted, in revised form, 16 July 1992)