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Intermediate filaments of human trophoblast and choriocarcinoma cell lines

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Summary. Human term placenta and two human choriocarcinoma cell lines were studied immunohistochemically and by immunoblotting with monoclonal antibodies to keratin polypeptides and vimentin. Four keratin polypeptides (40, 45, 52, 54 K) were detected in both normal and malignant trophoblastic cells. The presence of the 54 K keratin polypeptide distinguishes the benign and malignant trophoblastic cells from human embryonal carcinoma cells and a yolk sac carcinoma cell line.

Key words: Placenta – Keratin – Vimentin – Choriocarcinoma

Tropoblastic and yolk sac elements frequently are products of differentiation of human embryonal carcinoma cells and are thus developmentally closely related (Damjanov 1984). In a previous study we have shown (Damjanov et al. 1984) that human embryonal carcinoma cells express 3 keratin polypeptides (40, 45 and 52 K). We have now extended these studies to determine whether the keratins of trophoblastic cells and yolk sac carcinoma cells differ from those of typical embryonal carcinoma cells. Also since there are no data available on the keratin content of normal human placenta, we have immunoblotted the cytoskeletal proteins of term placenta.

Materials and methods

Tissues and cell lines. Human placentae were collected shortly post-partum from normal term deliveries. Malignant trophoblastic (choriocarcinoma) cell lines BeWo (Patillo and Gey 1968) and Jar (Patillo et al. 1971) and yolk sac carcinoma cell line 1411H (Vogelzang et al. 1983) were maintained in cell culture trough serial passaging in DMEM supplemented with 10% fetal bovine serum. Cells were collected for histochemistry and electrophoresis by gentle scraping with a rubber policeman followed by pipetting.

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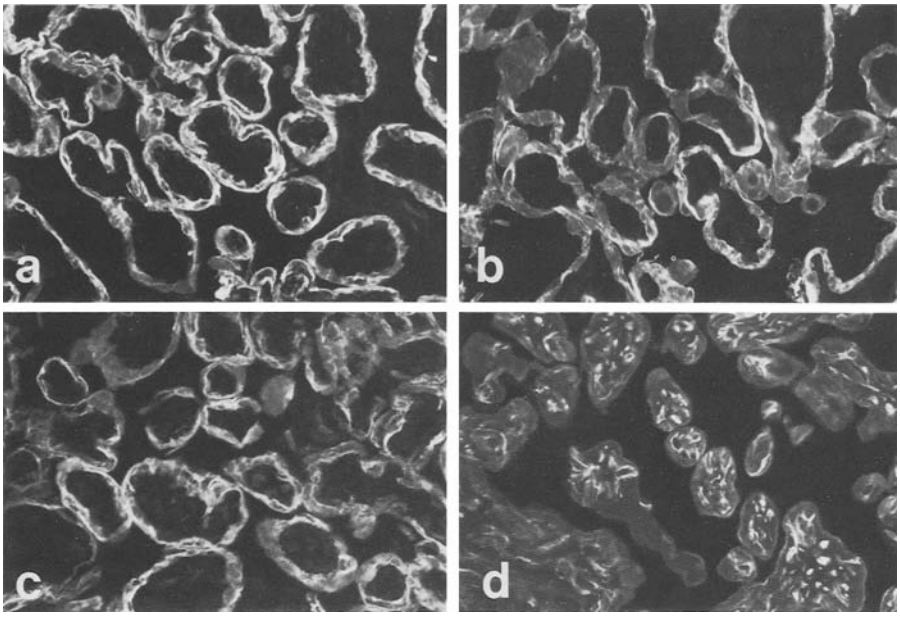

Fig. 1a–d. Frozen sections of normal human term placenta reacted with primary antibodies, followed by an FITC labeled secondary antibody. The following primary antibodies were used: **a** monoclonal antibody AE-1; **b** monoclonal antibody AE-3; **c** monoclonal antibody RGE-53; **d** polyvalent rabbit anti-vimentin antiserum

Histochemistry. Cryostat sections of snap frozen placenta and cytocentrifuge preparations of cell cultures were fixed in absolute acetone −20° C, for 10 min prior to incubation with the primary antibodies. Monoclonal antibodies AE-1, immunoreactive with most acidic keratins, AE-3 immunoreactive with all basic keratins (Sun et al. 1983 and 1984), Chrome-1 (Benham et al. 1983) and RGE-53 (Raemakers et al. 1983) both immunoreactive with 45 K keratin (number 18, Moll et al. 1982) and two polyvalent antisera produced in rabbits that were immunized with plantar callus keratins (donated by Dr. T.-T. Sun) or vimentin (donated by Dr. I. Virtanen) were layered over the tissues and cells overnight. The secondary antibodies were FITC-labeled goat anti-rabbit IgG, heavy and light chain specific. The tissue sections were covered with glycerine and the slides were examined on an Olympus microscope equipped with ultraviolet epiilumination.

Electrophoresis. One dimensional electrophoresis was performed on cytoskeletal extracts of cultured cell lines and placental tissues as previously described (Woodcock-Mitchell et al. 1982). Samples containing previously defined keratin polypeptides were run in parallel lanes as controls. The polypeptides were electrophoretically transferred to nitrocellulose membranes according to Towbin (1979). These blots were immunostained using the monoclonal antibodies described above as primary reagents, followed by a horseradish-peroxidase-labeled secondary antibody. The blots were developed in 3,3′-diaminobenzidine and 0.01% hydrogen peroxide, washed, dried and photographed as described previously (Woodcock-Mitchell et al. 1982).

Results

Normal placenta. Term placenta stained with all 4 monoclonal and polyclonal antibodies to keratin polypeptides showed identical reaction patterns.

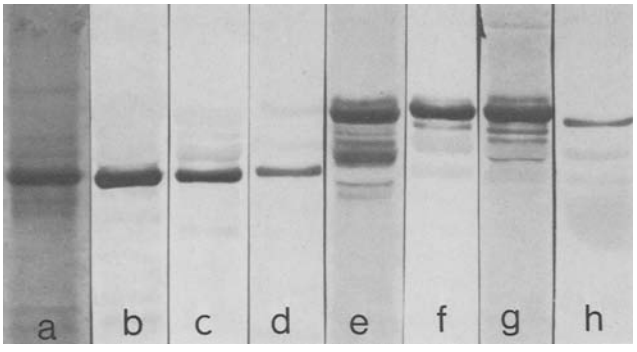

Fig. 2. Immunoblots of 10% SDS gels of cytoskeletal extracts from the following cells and tissues: lanes *a* and *e*, normal human term placenta; lanes *b* and *f*, choriocarcinoma cell line BeWo; lanes *c* and *g*, choriocarcinoma cell line Jar; lanes *d* and *h*, yolk sac carcinoma cell line 1411H. The following primary antibodies were used: lanes *a*, *b*, *c*, *d*, monoclonal antibody AE-1, lanes *e*, *f*, *g*, *h*, monoclonal antibody AE-3. The blots were treated with anti-mouse IgG horseradish peroxidase labeled secondary antibody and reacted with 3,3′-diaminobenzidine and 0.01% hydrogen peroxide. Immunoreactive bands can be seen in lanes *a* through *d* with an approximate molecular weight of 40 K, corresponding to keratin 19 (Moll et al. 1982). Lanes *e*, *f*, *g*, contain 2 immunoreactive bands with approximate molecular weights of 52 K and 54 K, corresponding to keratins 8 and 7 (Moll et al. 1982). Lane *h* contains one immunoreactive band with an approximate molecular weight of 52 K, corresponding to keratin 8 (Moll et al. 1982). The lower immunoreactive bands seen in lanes *a*, *e*, *f* and *g* are probably products of degradation of the above keratins

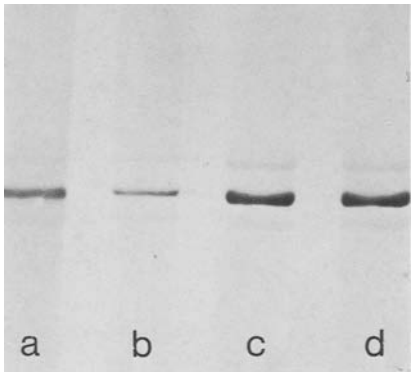

Fig. 3. An immunoblot of a 10% SDS gel of cytoskeletal extracts of the following cells and tissues: lane *a*, normal human term placenta; lane *b* choriocarcinoma cell line BeWo; lane *c*, choriocarcinoma line Jar; lane *d*, yolk sac carcinoma cell line 1411H. The immunoblot was incubated with monoclonal antibody Chrome-1, followed by an antimouse IgG horseradish peroxidase labeled secondary antibody. The blot was then reacted with 3,3′-diaminobenzidine and 0.01% hydrogen peroxide. Each lane contains one band with an approximate molecular weight of 45 K, corresponding to keratin 18 (Moll et al. 1982)

The cytoplasm of both cytotrophoblastic and syncytiotrophoblastic cells reacted strongly and a filamentous staining pattern was frequently discernable (Fig. 1). The antibody to vimentin reacted with stromal and vascular cells only. The immunoblots of the water insoluble cytoskeletal extracts disclosed 2 bands (52 and 54 K) immunoreactive with AE-3, corresponding to keratins 8 and 7 (Moll et al. 1982), 1 band (45 K) immunoreactive with Chrome-1 and RGE-53, corresponding to keratin 18 (Moll et al. 1982) and 1 band (40 K) immunoreactive with AE-1, corresponding to keratin 19 (Moll et al. 1982) (Figs. 2 and 3).

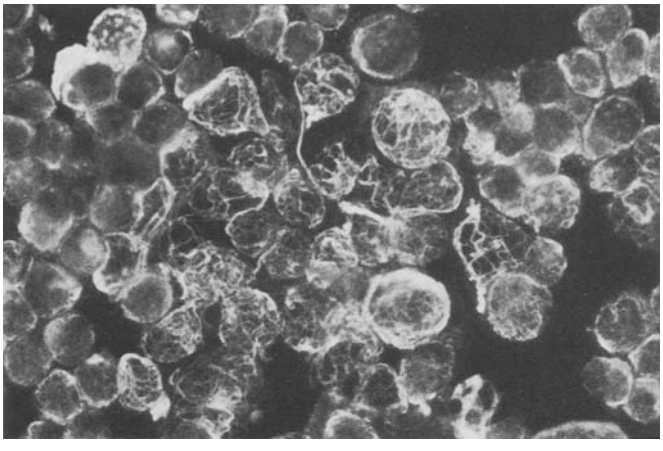

Fig. 4. Indirect immunofluorescence stained choriocarcinoma cell line BeWo reacted with polyvalent rabbit anti-keratin primary antiserum, followed by an FITC labeled antirabbit IgG secondary antibody

Choriocarcinoma cell lines. Most of the cells showed filamentous staining with either monoclonal or polyclonal antikeratin antibodies (Fig. 4). Immunoblots of BeWo and Jar cell cytoskeletal preparations showed reaction patterns indentical to those seen on the cytoskeletal extracts of normal placenta. Four distinct bands of 40, 45, 52 and 54 K were immunoreactive with the monoclonal antikeratin antibodies, corresponding to keratin's 19, 18, 8 and 7 (Moll et al. 1982) (Figs. 2 and 3).

Human yolk sac carcinoma. Line 1411H, which was derived from a teratocarcinoma and has the features of yolk sac carcinoma cells (Vogelzang et al. 1983) reacted with all anti-keratin antibodies. On immunoblotting the cytoskeletal extract contained three polypeptide bands: 52 K immunoreactive with AE-3 corresponding to keratin 8 (Sun et al. 1983), 54 K immunoreactive with Chrome 1 and RGE-53 corresponding to keratin 18 (Raemakers et al. 1983) and 40 K immunoreactive with AE-1 corresponding to keratin 19 (Tseng et al. 1982) (Figs. 2 and 3). Keratin 7 (Moll et al. 1982), the 54 K band, was not seen in extracts of 1411H. Human embryonal carcinoma cell line NT-2 (Andrews et al. 1984) exhibited the same 3 immunoreactive keratin bands (Damjanov et al. 1984) as the yolk sac carcinoma cell line.

Discussion

In the present study we have shown that trophoblastic cells of human placenta are simple epithelia and as such express the low molecular weight keratins typically found in many simple epithelia in the human body (Moll et al. 1982; Tseng et al. 1982; Wu et al. 1983; Osborn and Weber 1983; Cooper et al. 1985). It is of interest to note that both cytotrophoblastic and syncytiotrophoblastic cells reacted in the same manner, suggesting that

the differentiation of cytotrophoblastic cells into syncytiotrophoblast is not accompanied by a change in the IF cytoskeletal polypeptides.

While it has been shown that malignant alteration of stratified epithelial cells is often accompanied by changes in the keratins expressed (Nelson et al. 1984), it is also known that the keratin patterns of simple epithelia are generally stable upon malignant alteration (Franke et al. 1981; Moll et al. 1982 and 1983; Sun et al. 1984). The comparison of cytoskeletal IF polypeptides of normal human placenta and two gestational trophoblastic neoplasia derived cell lines indicates that these malignant cells have retained the full keratin polypeptide complement of their ancestral normal equivalents as would be predicted by their simple epithelial nature.

Trophoblastic cells are frequently found in mixed human germ cell tumors and it is generally accepted that these cells, together with yolk sac elements, represent some of the first differentiated descendants of embryonal carcinoma cells (Hochstetter et al. 1982). It is thus of interest to note that human embryonal carcinoma cells have the same 3 keratin polypeptides as the yolk sac carcinoma cell line. On the other hand, embryonal carcinoma and yolk sac carcinoma cells differ from the two choriocarcinoma lines, the latter exhibiting an additional 54 K polypeptide not found in any of the previously examined carcinoma cell lines (Damjanov et al. 1984). It remains to be determined whether this 54 K polypeptide occurs only in gestational choriocarcinomas, such as BeWo and Jar, or whether it is a universal marker of all trophoblastic cells. In that case the monoclonal antibodies reacting only with the 54 K keratin polypeptide would be a most useful reagent for immunohistochemical detection of trophoblastic cells in tumors, especially those that have not undergone transformation into syncytial giant cells and are thus indistinguishable from other mononuclear tumor cells in mixed nonseminomatous germ cell tumors or seminomas (Suurmeijer et al. 1982).

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