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Human Teratocarcinoma Stem Cells: Glycolipid Antigen Expression and Modulation During Differentiation

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Teratocarcinomas are germ cell tumors in which pluripotent stem cells, embryonal carcinoma (EC) cells, undergo differentiation along the pathways resembling those occurring during early embryogenesis. Human EC cell lines established in vitro provide a model for studying embryonic cellular differentiation in a way that is pertinent to early human development. The predominant glycolipid antigens expressd by EC cells of both humans and mice have globoseries core structures; in humans they are terminally modified to yield the monoclonal antibody-defined stage-specific embryonic antigens SSEA-3 and SSEA-4, and also globo-ABH antigens; in the mouse terminal modification yields the Forssman antigen rather than SSEA-3 and -4. These observations focus attention on the possible role of the P-blood group system, which regulates synthesis of globoseries oligosaccharides, in the behavior of cells in the early embryo and in teratocarcinomas. Marked changes in the core structures of the cell surface glycolipids occur as the EC cells differentiate; thus globoseries structures rapidly diminish and are replaced by lactoseries and then by ganglioseries glycolipids. During differentiation of the NTERA-2 line of pluripotent human EC cells into neurons and other cell types, the various subsets of differentiated cells that arise are distinguished by their differential expression of new glycolipid antigens, particularly ganglioside GT_3 (recognized by antibody A2B5), and ganglioside 9-0-acetyl GD_3 (recognized by antibody ME311). Neurons are found among the $A2B5^+/ME311^-$ cells.

Key words: human teratocarcinoma, embryonal carcinoma, glycolipids, antigens, cell surface, P-blood group, cell lines

Teratocarcinomas are heterogeneous tumors that contain seemingly haphazard collections of one or more tissues resembling those normally found elsewhere in the adult, developing embryo, or extra-embryonic membranes [1]. In addition, these tumors contain nests of cells, embryonal carcinoma (EC) cells, which resemble early embryonic cells and are considered to be the stem cells of the tumor [2]. Indeed, in teratocarcinomas of the mouse, it has been convincingly shown that differentiation of EC cells leads to all the other cell types in the tumor [3]. Moreover, when murine EC

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cells from some established lines are injected into a blastocyst, they take part in normal embryonic development leading to a chimeric adult mouse with tissues derived from the host blastocyst and from the injected EC cells [eg, 4,5]. Thus murine EC cells are thought to resemble cells of the embryonic primitive ectoderm in their developmental and biochemical properties, and their differentiation in culture has provided an important model for studying the molecular and cellular processes of early embryonic development [6,7]. Likewise human embryonal carcinoma should provide a model of cell differentiation with special relevance to early human development [8].

Teratocarcinomas are thought to arise from germ cells that have undergone malignant transformation. Related to teratocarcinomas are other so-called nonseminomatous germ cell tumors, embryonal carcinoma, yolk sac carcinoma, and choriocarcinomas, which may form components of teratocarcinomas but which can also occur in isolation [2,9]. Thus tumors may consist of EC cells alone, without any differentiated components. Such "nullipotent" EC cells appear to lack the capacity for differentiation of EC cells found in teratocarcinomas, but there are no other known distinguishing characteristics. Tumors composed only of somatic elements also occur and presumably arise by differentiation of pluripotent EC cells which subsequently disappear; such tumors are known as teratomas, the term "teratocarcinoma" being reserved for tumors containing an EC component. Yolk sac carcinomas and choriocarcinomas correspond to malignant cells with the properties of extraembryonic endoderm and trophoblast, respectively; they occur with or without an overt EC component and, again, presumably arise by differentiation of EC cells which may then be lost. Human germ cell tumors occur in the testes and ovaries, and also extragonadally, perhaps from misplaced germ cells that failed to complete their migration to the genital ridges during embryogenesis. Testicular germ cell tumors, in particular, show a peculiar age distribution with a peak incidence in the third decade of life; despite their overall rarity, testicular germ cell tumors are one of the most common forms of cancer in young adult males, and their incidence has been increasing during the past 20 to 30 years [eg, 10].

Many human cell lines derived from germ cell tumors have been established in culture and some have been shown to contain EC cells [see 11]. Investigation of these cell lines has begun to provide new objective criteria for recognizing the various cell types found in germ cell tumors, as well as ways of gaining insights into the properties of human embryonic cells [eg, see 12]. Recently we have investigated the differentiation of pluripotent EC cells in the TERA-2 cell line [13]. Differentiation of these EC cells, which results in the appearance of a variety of somatic cell types including neurons, is accompanied by many changes in cell phenotype. One of the most striking changes is the loss of glycolipid cell surface antigens characteristic of human EC cells and the appearance of new glycolipid antigens on the differentiated cells that arise. Recently, we have made a detailed study of these changes in the expression of glycolipid antigens as TERA-2 EC cells differentiate [14]. The results have clarified some earlier conflicting observations concerning the expression of globoseries antigens by EC cells and have indicated that some of the new antigens that appear are expressed by different subsets of differentiated cells. The data have also suggested that switching between the synthesis of different oligosaccharide core structures, regulated by a few key glycosyl transferases, may be an important event during cell differentiation in embryogenesis and perhaps also during oncogenesis.

HUMAN EC CELLS

Initial studies of human teratocarcinoma cells in culture were based on information gained using murine EC cell lines as a model, on the assumption that mouse and human cells are identical. However, it soon became clear that many differences existed between the apparently homologous tumor cells of these two species [15]. To clarify our understanding of human EC cells, we have since concentrated on two human cell lines, 2102Ep and TERA-2, derived from different testicular germ cell tumors and subsequently cloned by growing sublines from isolated single cells [16,17]. When injected into nude mice, cloned 2102Ep cells give rise to xenograft tumors histologically consistent with pure embryonal carcinoma. Thus 2102Ep was defined as a human EC cell line with limited capacity for differentiation. On the other hand, clonal lines isolated from TERA-2 give rise to xenograft tumors which, in addition to nests of EC cells, contain a variety of somatic cell types including neurons and glandular structures thought to represent primitive gut. Thus TERA-2 is a pluripotent EC cell line; it can also be induced to differentiate in vitro, for example, by exposure to retinoic acid or bromodeoxyuridine (BUdR) [13,18]. Most of our own work with TERA-2 EC cells has been conducted with a series of pluripotent clones isolated from a subline, NTERA-2, that was reestablished from a xenograft tumor grown in a nude mouse [17]. Other pluripotent EC clones have been isolated directly from TERA-2 without prior passage through a nude mouse, and several of these clones differ to some extent from more typical human EC cells [19,20], as discussed below.

When maintained under conditions of high cell density in vitro the cloned 2102Ep, TERA-2, and NTERA-2 cells exhibit a typical EC morphology (Fig. 1) and ultrastructure. These cells have a high nucleus:cytoplasm ratio, prominent nucleoli, and a nondescript cytoplasm containing few organelles other than free ribosomes [17,21]. These cells also express high levels of alkaline phosphatase activity, mainly of the liver isozyme, although lower levels of a placental-like isozyme are also detectable, especially in 2102Ep cells [22; see also 23]. These features are similar to those of murine EC cells and to those of other human EC cells growing in vitro and observed in clinical specimens of human germ cell tumors. On the other hand, 2102Ep and NTERA-2 EC cells differ from murine EC cells in that they express cytokeratin rather than vimentin intermediate filaments [21,24], and class I major histocompatibility complex (MHC) cell surface antigens (HLA-A, B, C, and β_2-microglobulin) [16,17]. Results obtained with other human germ cell tumor lines have confirmed that MHC antigens are indeed commonly expressed by human EC cells, albeit at a low level [25–28]. However, considerable variation in the level of expression is seen, especially by TERA-2 EC cells, among different subclones and even among different experiments with the same subclone; sometimes no MHC antigen expression has been observed [17,19,20,23]. However, we have recently reported that class I, but not class II, MHC antigens can be induced or augmented in human, and even in some murine, EC cells by interferon, especially interferon-gamma [29]. In addition to these widely expressed markers, several new surface antigens commonly expressed by human EC cells but few other cell types have been defined by monoclonal antibodies raised against germ cell tumor cell lines and serve as useful EC cell markers. These antigens include TRA-1-60 and TRA-1-81, defined by monoclonal antibodies raised to 2102Ep cells [30], and 5.H.1, 8.7.D, and 13.7.A defined by monoclonal antibodies

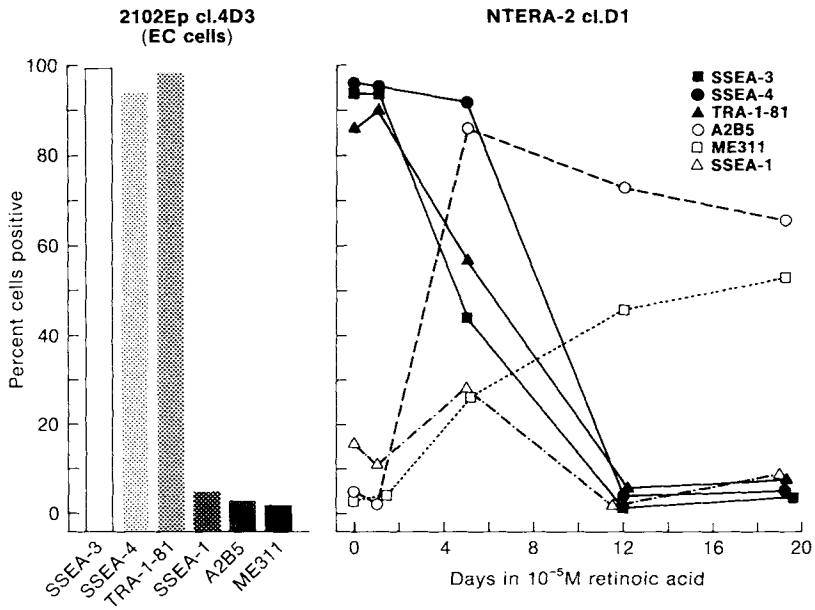

Fig. 1. The expression of glycolipid antigens SSEA-1, SSEA-3, SSEA-4, A2B5, and ME311 by 2102Ep and NTERA-2 cl.D1 human EC cells, and changes in the expression of these antigens during retinoic acid induced differentiation of NTERA-2 cl.D1 cells. The assay was by flow cytofluorimetry after binding of antibodies to live cells [see 17]. 2102Ep and NTERA-2 cl.D1 EC cells were from cultures seeded 3 to 4 days previously at 5×10^6 per 75 cm^2 flask; differentiation of NTERA-2 cl.D1 was induced by seeding EC cells at 10^6 per 75 cm^2 flask in medium containing 10^{-5} M retinoic acid [13].

raised to TERA-1 human teratocarcinoma cells [31]. Little is known of the molecular characteristics of these new markers, although several are associated with high molecular weight polypeptides; much more is now known of several glycolipid antigenic markers of human EC cells.

GLYCOLIPID MARKERS OF HUMAN EC CELLS

2102Ep EC cells strongly express the monoclonal antibody-defined murine stage-specific embryonic antigens SSEA-3 and SSEA-4 but not the embryonic antigen SSEA-1 (Fig. 1) [16,32]. By contrast, murine EC cells generally express SSEA-1 but not SSEA-3 or SSEA-4. This accords with the view that murine EC cells are developmentally equivalent to the primitive ectoderm of the embryo [6], cells of which are also SSEA-1^+ but SSEA-3^- and SSEA-4^- [32–34]. On the other hand, both SSEA-3 and SSEA-4 are expressed on cleavage-stage mouse embryos, when SSEA-1 is not expressed. This suggested the idea that human EC cells may be developmentally equivalent to an embryologically earlier cell type than murine EC cells [8,15]. Results with other cell lines and with clinical specimens of germ cell tumors have confirmed that the SSEA-1^-/SSEA-3^+/SSEA-4^+ phenotype is indeed common amongst human EC cells [34,35].

Like other human EC cells, the NTERA-2 EC cells, as well as some EC cells in the parental TERA-2 line and clones isolated directly from it, also express SSEA-3 and -4, but not SSEA-1 [14,17,19]. Nevertheless, some clones of EC cells isolated

directly from TERA-2 completely lack cell surface expression of SSEA-3 and -4 when tested by antibody binding assays to whole cells, although they resemble the NTERA-2 EC cells morphologically and in their capacity for forming well-differentiated tumors in nude mice [19,20]. Curiously, however, when one such SSEA-3^-/4^- clone, TERA-2 cl.w1, was injected into nude mice, the EC cells that grew in the resulting tumors *did* express both SSEA-3 and -4. Moreover, when these tumors were explanted in vitro, the EC cells that grew out continued to express these antigens, and such SSEA-3^+/4^+ EC cells persisted for several months, even when contaminating mouse fibroblasts derived from the xenograft host were no longer detectable [19]. The significance of these results, and the mechanisms whereby tumor growth can switch on the expression of SSEA-3 and -4, remains unclear. Nevertheless, the observations suggest a relationship between the growth of EC cells in tumors and their expression of SSEA-3 and -4. Since SSEA-3 and -4 are antigens that belong to the polymorphic P-blood group system (see below), the question arises of whether the P-blood group genotype may influence the behavior of germ cell tumors in patients.

The SSEA-1, SSEA-3, and SSEA-4 epitopes are all carried on glycosphingolipids (Table I). The SSEA-1 epitope is composed of Fuc(α1→3)GlcNAc carried by a type 2 polylactosamine chain of a lactoseries glycolipid [36,37]. On the other hand SSEA-3 and SSEA-4 are both extended globoseries structures [32,38] (Table I); the antibody defining SSEA-3 recognizes the internal oligosaccharide sequence, whereas the antibody defining SSEA-4 recognizes the terminal structure and reactivity is dependent upon the terminal sialic acid. The globoseries glycolipids also contain epitopes recognized as red blood cell antigens of the P-blood group system; the P antigen is associated with globoside (Gb_4), whereas the P^k antigen is associated with Gb_3 [39]. Accordingly, P and P^k antigens have been detected on several human teratocarcinoma cell lines [40], while SSEA-3 and -4 are expressed on red blood cells. As expected, rare individuals with p and P^k phenotypes, who lack the glycosyl transferases necessary for extending the globoseries chain from lactosyl ceramide and Gb_3, respectively, and so do not express the P antigen, also do not express either SSEA-3 or -4 on their red blood cells [41]. In addition, red blood cells from about 1% of people who express P and SSEA-3 do not express SSEA-4, which appears to correspond the previously described Luke antigen, now named LKE [41]. Since these globoseries antigens are expressed on human EC cells as well as embryonic mouse cells, they are probably also expressed on early human embryos. Thus the high rates of spontaneous abortion in women with the p phenotype (ie, who lack expression of the P antigen and of SSEA-3 and -4) could be due to an anti-embryo immune response in such individuals directed to any of the P, SSEA-3, or SSEA-4 epitopes [42]; the p allele is both very rare and recessive so that embryos of women with the p phenotype will almost always have a *P/p* genotype and be capable of expressing these antigens. Our results now raise the question of whether the much more common LKE(−) women might also experience high rates of spontaneous abortion owing to an anti-embryo immune response directed to the SSEA-4 epitope; this remains to be addressed.

When analyzed by thin-layer chromatography and immunostaining, undifferentiated 2102Ep and NTERA-2 EC cells were found to contain predominantly globoseries glycolipids, including Gb_3, Gb_5, Bl_7, globe-H, and in NTERA-2, globo-A structures (Table I) [14,32,38]. The presence of globo-H and globo-A as the major

TABLE I. Glycosphingolipid Antigens of Human Teratocarcinoma Cells*

Glycolipid/antigen [reference]	Glycolipid structure
Globo-A [14]	Fucα1→2Galβ1→3GalNAcβ1→3Galα1→**4Galβ1→4G1cβ1→Cer** 3 GalNAcα1↑
Globo-H [14,32,38]	Fucα1→2Galβ1→3GalNAcβ1→3Galα1→**4Galβ1→4G1cβ1→Cer**
Gl_7,SSEA-3, SSEA-4 [14,32,38]	NeuAcα2→3Galβ1→3GalNAcβ1→3Galα1→**4Galβ1→4G1cβ1→Cer**
Gb_5,SSEA-3 [32]	Galβ1→3GalNAcβ1→3Galα1→**4Galβ1→4Glcβ1→Cer**
Gb_4,SSEA-3, P [32,39]	GalNAβ1→3Galα1→**4Galβ1→4Glcβ1→Cer**
Gb_3,P^k [39]	Galα1→**4Galβ1→4Glcβ1→Cer**
Le^x, SSEA-1 [36,37]	Galβ1→4GlcNAcβ1→**3Galβ1→4Glcβ1→Cer** 3 Fucα1↑
Galβ1→4Glcβ1→Cer	lactosyl ceramide
GD_3 [14]	NeuAcα2→8NeuAcα2→**3Galβ1→4Glcβ1→Cer**
GT_3, A2B5 [14,50]	NeuAcα2→8NeuAcα2→8NeuAcα2→**3Galβ1→4Glcβ1→Cer**
(9-0-acetyl)GD_3, ME311 [14,51]	(9-0-acetyl)NeuAcα2→8NeuAcα2→**3Galβ1→4Glcβ1→Cer**

a, b, c

*Key glycosyl transferases, postulated to regulate core structure switching: a) α(1→4)galactosyl transferase; b) α(1→3)N-acetylglucosaminyl transferase; c) α(2→3)sialyltransferase.

blood group ABH antigens in EC cells suggests that these are embryonic antigens, synthesis of which switches to lactoseries during later stages of human development. Analysis of EC cell lines derived from patients of known ABO blood type indicated that the globo-ABH antigens are probably under the same genetic control as the lactoseries ABH antigens of adult red blood cells [14]. Globoseries glycolipids also predominated in TERA-2 cl.wl EC cells, despite their lack of surface reactivity with antibodies to SSEA-3 and -4 [19]. However, the globoseries glycolipid Gl_7 was not observed, whereas it was detected in all the SSEA-3^+ and -4^+ EC cells. This suggests that Gl_7 is the immunodominant structure on the surface of human EC cells responsible for the reactivity of whole cells with anti-SSEA-3 as well as anti-SSEA-4. The reason for the absence of Gl_7 from TERA-2 cl.wl EC cells is not known.

Consideration of the foregoing results with human EC cells, and of previous studies of glycolipids in mouse EC cells suggests that the distinction between the EC cells of these two species is not as marked as originally thought [eg, see 8]. Thus although the lactoseries antigen SSEA-1 is readily detectable on the surface of mouse EC cells [33,37], the predominant glycolipids present in mouse EC cells, as in human EC cells, are globoseries glycolipids [43,44]. However, instead of being modified to yield SSEA-3- and-4-reactive structures, they are modified to yield the Forssman antigen. The distinction is thus in the terminal rather than in the core structure of the predominant glycolipids. Although most human EC cells generally express little or no SSEA-1, several of the TERA-2 cloned lines do express higher levels than usual, but not as high as in murine EC cells. As discussed below, lactoseries glycolipids such as SSEA-1 appear soon after the differentiation of (SSEA-1^-) human EC cells is induced. Thus, the presence of lactoseries antigens in otherwise undifferentiated EC cells may indicate progression to an early differentiated state in which a developmental program of oligosaccharide core switching has been initiated without other (later) changes usually associated with the complete loss of an EC phenotype. Such a notion might still imply that most (SSEA-1^-) human EC cells correspond to an earlier state of embryogenesis than do murine EC cells. Even so, the significance of SSEA-1 expression by murine EC cells is unclear since variant SSEA-1^- murine EC cells have been selected and do not otherwise differ detectably from their parental SSEA-1^+ EC cells in their ability to form tumors or to differentiate in vitro [45].

DIFFERENTIATION OF HUMAN EC CELLS

2102Ep EC cells maintained at a high cell density retain an EC phenotype. However, when seeded at a low cell density, many of the cells acquire a larger, flatter morphology and begin to express SSEA-1; expression of SSEA-3 is concomitantly reduced [16]. The SSEA-1^+ cells also start to synthesize and secrete fibronectin [46], which is of slightly larger molecular weight than fibroblast fibronectin and is differently glycosylated with covalently linked heparan sulfate and lactosaminoglycans [47]. Such limited, low-density-induced differentiation seems to be common among several human EC cell lines, including TERA-2 (Fig. 2), in culture [eg, 15,17,23,48]. However, many EC cells often persist, and the relationship of the differentiated cells that arise to embryonic cells or to cells seen in tumors in vivo is unknown.

By contrast, retinoic acid induces the almost complete differentiation of cloned NTERA-2 EC cells into a variety of cells including neurons [13,49]. Within about 7 days of seeding in 10^{-5} M retinoic acid NTERA-2 cells lose their typical EC

morphology and growth characteristics. In the subsequent 7–14-day period, many cells expressing 70, 170, and 200-kilodalton (kD) neurofilament polypeptides appear, and some of these develop a typical neuronal morphology (Figs. 2, 3). Immunofluorescence analysis indicates the disappearance of cells expressing both SSEA-3 and -4 over this same period (Fig. 1). Oddly, SSEA-3 appears to be lost faster than SSEA-4 even though anti-SSEA-3 recognises the immunodominant glycolipid Gl_7, with which anti-SSEA-4 also reacts, and which is detectable in the cells for several days longer than surface reactivity with anti-SSEA-3. Immunochemical analysis of the glycolipids extracted from the differentiating cells suggests that this apparent paradox rests in the fact that certain gangliosides (eg, GD_3), induced during differentiation, inhibit the binding of anti-SSEA-3 to Gl_7 even though they do not contain the SSEA-3 epitope [14]. The mechanism of this inhibition remains obscure, but the observation serves to emphasize the lack of correlation, also reported by others [eg, 43], between the presence of a glycolipid antigen in the cell surface and the ability to detect it by immunobinding assays to whole cells. Nevertheless, thin layer chromatographic analysis of glycoplipids isolated from differentiating NTERA-2 cells does confirm the marked decrease in the synthesis of globoseries structures during the 2–3 weeks following the initiation of differentiation with retinoic acid.

As the globoseries glycolipids characteristic of EC cells disappear, new glycolipids with lactoseries and ganglioseries core structures appear [14]. The lactoseries glycolipids appear early and only transiently, with peak SSEA-1 expression after about 7 days and a decline thereafter. Many of these SSEA-1^+ cells are found in isolated clusters, seemingly forming a group distinct from the SSEA-1^- cells present at 7 days (Fig. 3). At this time most of the cells express the newly induced antigen A2B5 [50], which is carried as the GT_3 ganglioside in these cultures [14]. Another ganglioside antigen, ME311, identified as 9-O-acetyl GD_3 [51], also appears but on

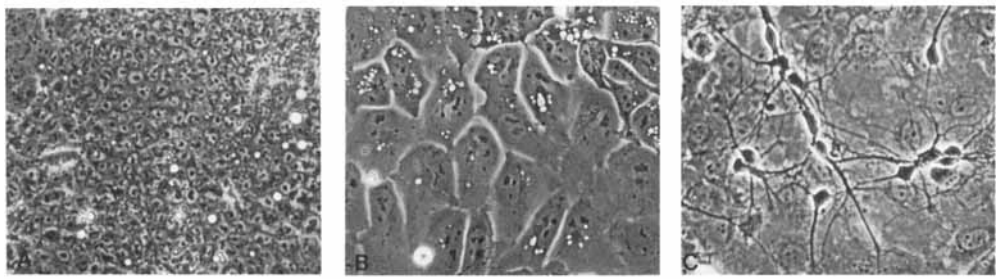

Fig. 2. Morphology of NTERA-2 cells grown at (**A**) high density (cells seeded at 5×10^6 cells per 75 cm^2 flask) and (**B**) low density (cells seeded at 10^5 cells per 75 cm^2 flask). The cells at high density exhibit a typical EC morphology; the change in morphology at low density is accompanied by changes in biochemical and antigenic characteristics as discussed in the text. **C:** Neuronlike cells in a differentiated culture of NTERA-2, induced with retinoic acid [13].

Fig. 3. Immunofluorescence staining of differentiated NTERA-2 cl.D1 cells growing on glass coverslips. **a,b:** Two-color immunofluorescence staining of the same field for neurofilaments (a) and surface expression of A2B5 (b). **c,d:** Two-color immunofluorescence staining of the same field for neurofilaments (c) and surface expression of ME311 (d). Note that neurofilament-positive cells with a neuronal morphology express A2B5; neurofilament-positive cells without a neuronal morphology do not express either A2B5 (not shown) or ME311 (d). **e:** Island of SSEA-1^+ cells; the remaining field contains confluent SSEA-1^- cells.

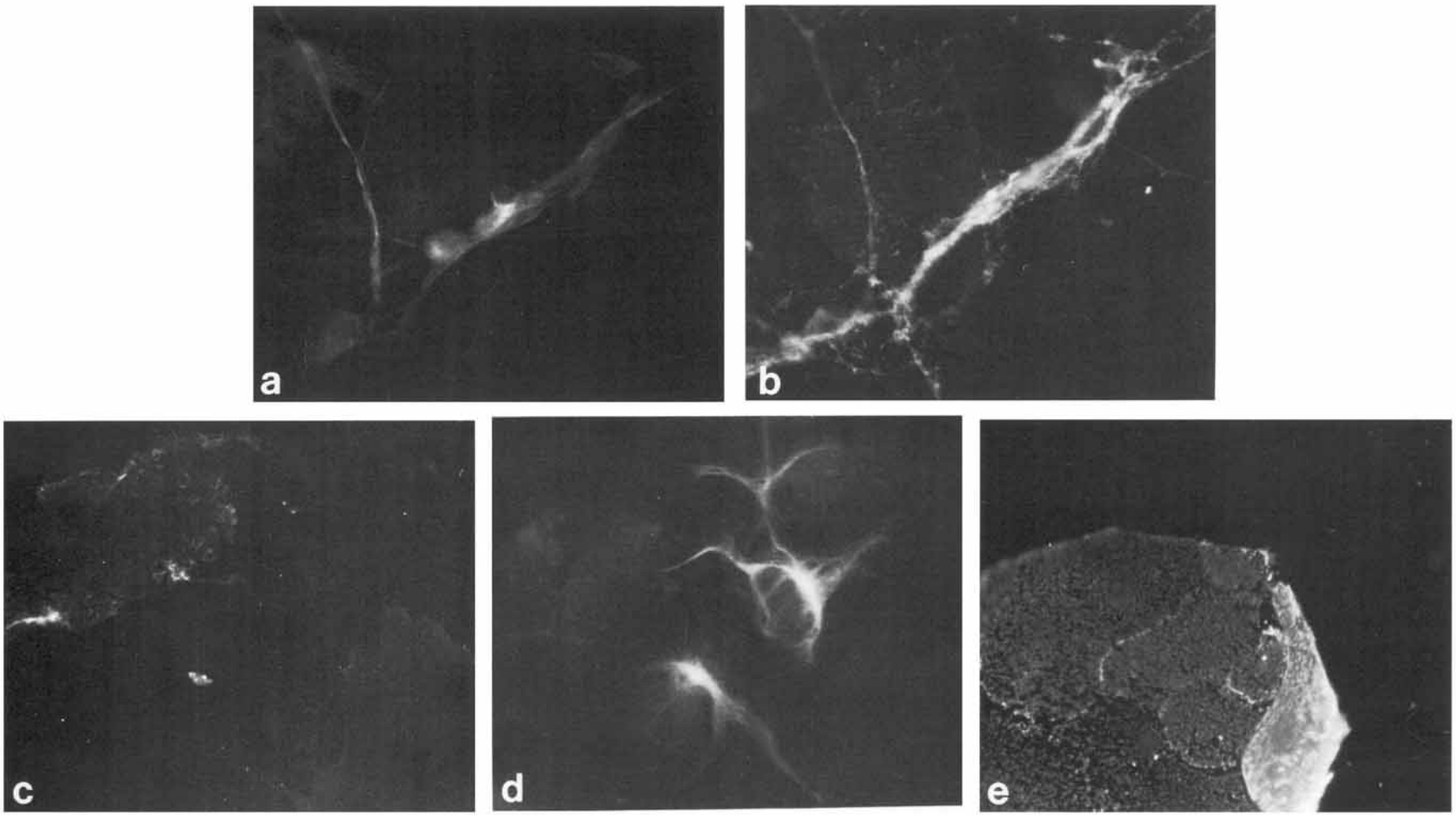

Figure 3

fewer cells. During this early phase of NTERA-2 differentiation, most cells are either $A2B5^+/ME311^+$ or $A2B5^+/ME311^-$; few are $A2B5^-$. Later the $A2B5^+$ and $ME311^+$ cells appear to diverge into two distinct lineages—the neurons are found among the $A2B5^+/ME311^-$ cells and not among the $ME311^+$ cells. Interestingly, the first neurofilament-expressing cells to appear between 7 and 14 days express neither A2B5 nor ME311 at this stage; nor do they express SSEA-1. The precise lineage relationships among the EC stem cells, the neurons, and the various subsets of differentiated cells defined by their surface antigen phenotype is the subject of current studies.

These results, derived by both thin layer chromatographic analysis of isolated glycolipids and by one- and two-color immunofluorescence analysis of the distribution of antigens among the differentiating cells, suggest that many of the changes in surface antigen expression during NTERA-2 EC cell differentiation could be explained by the regultion of a few key glycosyltransferases which initiate the synthesis of globo-, lacto-, and ganglio-core structures from lactosyl ceramide, the common precursor of all these glycolipids. However, modulation of some terminal structures must also be hypothesized; eg, A2B5 (GT_3) and ME311 (9-O-acetyl GD_3) presumably arise by sialylation or acetylation of a common precursor, GD_3, in different subsets of differentiated NTERA-2 cells, at least at later times after retinoic acid-initiated differentiation.

CONCLUSIONS

Studies of human teratocarcinoma cell lines in vitro have permitted the identification of EC cell lines that are able to differentiate to greater or lesser extents, and have allowed us to define various objective EC cell markers that provide new criteria for recognising these cells, both in vivo and in vitro. Prominent among these markers are the globoseries glycolipid antigens which, as a group, appear to be expressed early in embryonic development. Our observations suggest that further investigation of the role of these glycolipids in embryonic development and in the behavior of germ cell tumors in patients, and of the possible effects of different alleles of the P-blood group system which controls globoseries carbohydrate synthesis, is warranted. The results with differentiating NTERA-2 EC cells also focus attention on changes in the synthesis of cell surface carbohydrates and the role that glycosyl transferases, particularly those that effect switching between different core structures, may have on regulating cellular differentiation, and perhaps morphogenetic cellular interactions, in both embryogenesis and oncogenesis.

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REFERENCES

1. Stevens LC: Adv Morphol 6:1, 1967.
2. Friedman NB, Moore RA: Milit Surgeon 99:573, 1946.
3. Kleinsmith LJ, Pierce GB: Cancer Res 24:1544, 1964.
4. Brinster RL: J Exp Med 140:1049, 1974.
5. Mintz B, Illmensee K: Proc Natl Acad Sci USA 72:3585, 1975.
6. Solter D, Damjanov I: Methods Cancer Res 18:277, 1979.
7. Martin GR: Science 209:768, 1980.
8. Andrews PW, Goodfellow PN, Damjanov I: Cancer Surveys 2:41, 1983.
9. Damjanov I: In Damjanov I, Knowles BB, Solter D (eds): "The Human Teratomas; Experimental and Clinical Biology." Clifton, NJ: Humana Press, 1983, pp 23–66.
10. Osterlind A: Br J Cancer 53:501, 1986.
11. Andrews PW: In Damjanov I, Knowles BB, Solter D (eds): "The Human Teratomas; Experimental and Clinical Biology." Clifton, NJ: Humana Press, 1983, pp 285–311.
12. Andrews PW, Damjanov I: In Sell S, Reisfeld RA (eds): "Monoclonal Antibodies in Cancer." Clifton, NJ: Humana Press, 1985, pp 339–364.
13. Andrews PW: Dev Biol 103:285, 1984.
14. Fenderson BA, Andrews PW, Nudelman E, Clausen H, Hakomori S-i: Dev Biol 122:21, 1987.
15. Andrews PW, Bronson DL, Benham F, Strickland S, Knowles BB: Int J Cancer 26:269, 1980.
16. Andrews PW, Goodfellow PN, Shevinsky L, Bronson DL, Knowles BB: Int J Cancer 29:523, 1982.
17. Andrews PW, Damjanov I, Simon D, Banting G, Carlin C, Dracopoli NC, Fogh J: Lab Invest 50:147, 1984.
18. Andrews PW, Gönczöl E, Plotkin SA, Dignazio M, Oosterhuis JW: Differentiation 31:119, 1986.
19. Andrews PW, Damjanov I, Simon D, Dignazio M: Differentiation 29:127, 1985.
20. Thompson S, Stern PL, Webb M, Walsh FS, Engstrom W, Evans EP, Shi WK, Hopkins B, Graham CF: J Cell Sci 72:37, 1984.
21. Damjanov I, Andrews PW: Cancer Res 43:2190, 1983.
22. Benham FJ, Andrews PW, Bronson DL, Knowles BB, Harris H: Dev Biol 88:279, 1981.
23. Cotte C, Raghavan D, McIlhinney RAJ: In Vitro 18:739, 1982.
24. Damjanov I, Clark CK, Andrews PW: Cell Differ 15:133, 1984.
25. Bronson DL, Andrews PW, Solter D, Cervenka J, Lange PH, Fraley EE: Cancer Res 40:2500, 1980.
26. Andrews PW, Bronson DL, Wiles MV, Goodfellow PN: Tissue Antigens 17:493, 1981.
27. Avner P, Bono R, Berger R, Fellous M: J Immunogenet 8:151, 1981.
28. Morello D, Avner P: Ann Immunol (Inst Pasteur) 133:121, 1982.
29. Andrews PW, Trinchieri G, Perussia B, Baglioni C: Cancer Res 47:740, 1987.
30. Andrews PW, Banting GS, Damjanov I, Arnaud D, Avner P: Hybridoma 3:347, 1984.
31. Blaineau C, Connan F, Arnaud D, Andrews PW, Williams L, McIlhinney RAJ, Avner P: Int J Cancer 34:487, 1984.
32. Kannagi R, Cochran NA, Ishigami F, Hakomori S-i, Andrews PW, Knowles BB, Solter D: EMBO J 2:2355, 1983.
33. Solter D, Knowles BB: Proc Natl Acad Sci USA 75:5565, 1978.
34. Shevinsky LH, Knowles BB, Damjanov I, Solter D: Cell 30:697, 1982.
35. Damjanov I, Fox N, Knowles BB, Solter D, Lange PH, Fraley EE: Am J Pathol 108:225, 1982.
36. Gooi HC, Feizi T, Kapadia A, Knowles BB, Solter D, Evans MJ: Nature 292:156, 1981.
37. Kannagi R, Nudelman E, Levery SB, Hakomori S-i: J Biol Chem 257:14865, 1982.
38. Kannagi R, Cochran NA, Ishigami F, Hakomori S-i, Shevinsky LH, Knowles BB, Solter D: J Biol Chem 258:8934, 1983.
39. Naiki M, Marcus DM: Biochem Biophys Res Commun 60:1105, 1974.
40. Bono R, Cartron JP, Mulet C, Avner P, Fellous M: Blood Transfusion Immunohematol 24:97, 1981.
41. Tippett P, Andrews PW, Knowles BB, Solter D, Goodfellow PN: Vox Sang 51:53, 1986.
42. Race RR, Sanger, R: "Blood Groups in Man," 6th ed. Oxford: Blackwell Scientific Publications, 1975.
43. Willison KR, Karol RA, Suzuki A, Kundu SK, Marcus DM: J Immunol 129:603, 1982.
44. Carroll PG, Dewolf WC, Schwarting GA: In Silver LM, Martin GR, Strickland S (eds): "Teratocarcinoma Stem Cells." Cold Spring Harbor, NY: Cold Spring Harbor Conferences on Cell Proliferation, Vol. 10, 1983, pp 615–622.

45. Rosenstraus MJ: Dev Biol 99:318, 1983.
46. Andrews PW: Int J Cancer 30:567, 1982.
47. Cossu G, Andrews PW, Warren L: Biochem Biophys Res Commun 111:952, 1983.
48. McIlhinney RAJ, Patel S, Monaghan P: Exp Cell Res 144:297, 1983.
49. Lee VM-Y, Andrews PW: J Neurosci 6:514, 1986.
50. Eisenbarth GS, Walsh FS, Nirenberg M: Proc Natl Acad Sci USA 76:4913, 1979.
51. Thurin J, Herlyn M, Hindsgaul O, Strömberg N, Karlsson KA, Elder D, Steplewski Z, Koprowski H: J Biol Chem 160:14556, 1985.