

## Thomsen–Friedenreich and its Precursor (Tn) Antigen Expression in Normal Skin and in Benign Cutaneous Tumours: A Marker for Sebaceous Differentiation

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The Thomsen–Friedenreich (T) antigen is the core disaccharide of cancer-associated carbohydrates, whose expression allegedly correlates with the prognosis of some carcinomas. We studied the expression of the T antigen and its precursor (Tn) with monoclonal antibodies in formalin-fixed specimens of normal skin and various benign cutaneous tumours and inflammatory lesions (n: 105). In normal skin, both antigens were consistently expressed within the cytoplasm of mature sebocytes and rarely over the luminal surface of secretory sweat gland cells. All (21/21) sebaceous tumours showed strong T/Tn positivity; several (9/16) sweat-gland tumours were also immunoreactive, although more weakly. Pilar (n=11), non-adnexal tumours (n=45) and inflammatory lesions (n=12) were as a rule unreactive. These results suggest that the T antigen is a sensitive marker of sebaceous differentiation that can be used for the study of adnexal skin tumours in routinely processed tissue specimens. **Key words:** Thomsen–Friedenreich antigen; T/Tn antigen; sebaceous glands; sebaceous tumours; sweat gland tumours.

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The Thomsen–Friedenreich (T) antigen (Gal  $\beta$ (1-3)GalNac  $\alpha$ (1-O)-Ser/Thr) and its precursor the Tn antigen (GalNac  $\alpha$ (1-O)-Ser/Thr) are the core disaccharides of O-glycosylated complex carbohydrates presumed to be cancer-associated antigens (1). Indeed, whereas in normal tissues these antigens are cryptic (sialylated), detected only after treatment with sialidase, they are expressed in carcinomas. Interestingly, their expression has been found in some cancer types (breast, cervix uterus, urinary bladder) to correlate with tumour aggressiveness and prognosis (2–5). The detection of these antigens has been performed in the past with lectins. More recently, specific monoclonal antibodies have become available that can be used for the immunohistochemical detection of T, Tn and related histo-blood group antigens. Up till now few data exist concerning the distribution of T/Tn antigens detected with monoclonal antibodies in normal and neoplastic human skin (6, 7). The potential implication of these antigens in tumoural pathology prompted us to investigate the expression of T/Tn antigens in normal human skin and in a variety of cutaneous tumours.

### MATERIAL AND METHODS

#### Tissue samples

Tissue samples included specimens of normal skin obtained through plastic surgery and biopsy or excision specimens of various benign cutaneous tumours (48 adnexal and 45 non-adnexal). Twelve specimens of various inflammatory dermatoses were also studied (Table I).

The material had been collected in our dermatopathology laboratory over the past 4 years, formalin-fixed and paraffin-embedded.

#### Immunohistochemistry

Four  $\mu$ m-thick sections placed on clean glass slides were deparaffinized and rehydrated, then immunolabelled according to a streptavidin-biotin-peroxidase technique (kit LSAB Dako, Copenhagen, Denmark), including the following steps: (a) inhibition of endogenous peroxidase with 1% H<sub>2</sub>O<sub>2</sub> in PBS; (b) incubation of the sections with blocking (non-immune) serum; (c) incubation with the primary antibodies for 15 min in moist chambers at room temperature. Primary antibodies included: (i) clone HB-T1, a mouse IgMk anti-T antigen produced against the terminal  $\beta$  Gal-1-3  $\alpha$  GalNac carrying glycosphingolipid isolated from human blood group A erythrocytes, and (ii) clone HB-Tn1, a mouse IgMk produced with asialo-ovine submaxillary mucin as immunogen and recognizing the Tn antigen (8). Both reagents were purchased from Dako (Copenhagen, Denmark) and used at a dilution of 1:40; (d) incubation with biotin-conjugated antiserum to mouse immunoglobulins (10 min); (e) incubation with peroxidase-conjugated streptavidin (10 min). The reaction was revealed with aminoethylcarbazole as chromogen. The intensity of immunolabelling was graded as follows: 0 (absent),  $\pm$  (weak), + (moderate), ++ (strong). Negative controls were performed by omitting the first layer antibody and proved consistently negative.

### RESULTS

#### Normal human skin

In all specimens studied, the T-antigen was found expressed in the cytoplasm of mature sebocytes, the basal ones remaining

Table I. Expression of T $\gamma$ /Tn antigens in adnexal tumours

Diagnosis	Total no.	T+	Tn+
Sebaceous tumours	21	21	21
Naevus sebaceous (Jadassohn)	13	13/++	13/+
Sebaceous hyperplasia (senile)	6	6/++	6/+
Sebaceous adenoma	1	1/ $\pm$	1/ $\pm$
Sebocystomatosis	1	1/++	1/+ +
Pilar tumours	11	0*	0*
Follicular poroma	4	0	0
Pilomatricoma	2	0	0
Trichofolliculoma	1	0	0
Trichilemmoma	1	0	0
Basaloid follicular hamartoma	1	0	0
Epidermoid cyst	2	0	0
Sweat-gland tumours	16	9	7
Hydrocystoma	4	3/+	3/+
Syringomas	3	0	0
Hidradenoma papilliferum	2	2/+	2/+
Syringocystadenoma papilliferum	1	0	1/+
Chondroid syringoma	2	2/+	1/ $\pm$
Nodular hidradenoma	2	1/ $\pm$	0
Eccrine poroma	1	1/ $\pm$	0
Eccrine spiradenoma	1	0	0

\* Except from normal sebaceous glands present in the section.

unreactive (Fig. 1A). The percentage of sebocytes stained in each specimen varied between 60 and 90%. The labelling had a granular appearance, with occasionally a perinuclear and cell-membrane intensification. In comparison, Tn antigen immunoreactivity was seen within the more mature sebocytes, i.e. those lying close to the excretory duct (Fig. 1B). In each gland fewer sebocytes (20–60%) were Tn-positive than were T-positive. The staining intensity was also weaker, with the immunoreactivity sometimes confined to the perinuclear space. T and Tn antigen immunoreactivity was rarely seen at the apical pole of cells of the secretory coil and the proximal excretory duct of eccrine and apocrine sweat glands (Fig. 1G). All other skin components (epidermal keratinocytes, acrosyringia and the epithelial sheath of hair follicles) were unreactive. Remarkably, *Demodex folliculorum* mites found within hair follicles in specimens of facial skin were strongly immunoreactive with the antibody to the Tn (but not to the T) antigen.

#### *Cutaneous tumours (Tables I and II)*

Consistent T/Tn immunoreactivity was seen in all 21 proliferative lesions with *sebaceous differentiation*, including naevus sebaceous of Jadassohn (Figs. 1C–D), senile sebaceous hyperplasia, sebaceous adenoma and sebocystomatosis (Figs. 1E–F). In these cases, the labelling was reminiscent of that observed in normal sebaceous glands, i.e. cells with sebaceous differentiation expressed stronger T than Tn immunoreactivity, with the ratio of T/Tn cells in the range 1.5–2:1. No obvious differences were seen among naevoid conditions (e.g. sebaceous nevus of Jadassohn) and acquired proliferations (e.g. senile sebaceous hyperplasia). The staining (for the T antigen) was sensitive, allowing the easy visualization of isolated sebaceous cells. In a single case of sebaceous nevus T (but not Tn) antigen immunoreactivity was seen within scattered cells of the basal epidermal cell layer (Fig. 1C). T/Tn immunoreactivity was also detected in a sizeable proportion (9/16) of *sweat-gland neoplasms* of alleged eccrine and apocrine differentiation, namely those with an excretory component (such as hidradenoma papilliferum) (Fig. 1H). In these cases the staining pattern was reminiscent of that seen in normal sweat glands, i.e. it was present over the apical pole of cells lining lumina, different from the cytoplasmic pattern seen in sebaceous glands and proliferations. *Pilar tumours* were completely unreactive (with the exception of sebaceous cells present within or in the vicinity of the tumour). *Non-adnexal epithelial or mesenchymal proliferations* were as a rule unreactive. In one case of each of dermatitis, lichen planus and clear-cell acanthoma weak T (but not Tn) antigen expression was found within the upper epidermal layer keratinocytes. Conversely, weak Tn (but not T) antigen cytoplasmic reactivity was found in one case of benign naevus and granular-cell tumour.

#### DISCUSSION

The T antigen and its precursor the Tn antigen are complex mucin-type carbohydrate antigens whose expression has been studied in visceral carcinomas and has been found in some cases to correlate with their course (1–5). These antigens can be recognized with some plant lectins (*Arachis Hypogaea*-PNA and *Vicia Villosa* Agglutinin-VVA, respectively), but these reagents are less specific, as shown by their much wider reactivity pattern than that obtained with the monoclonal

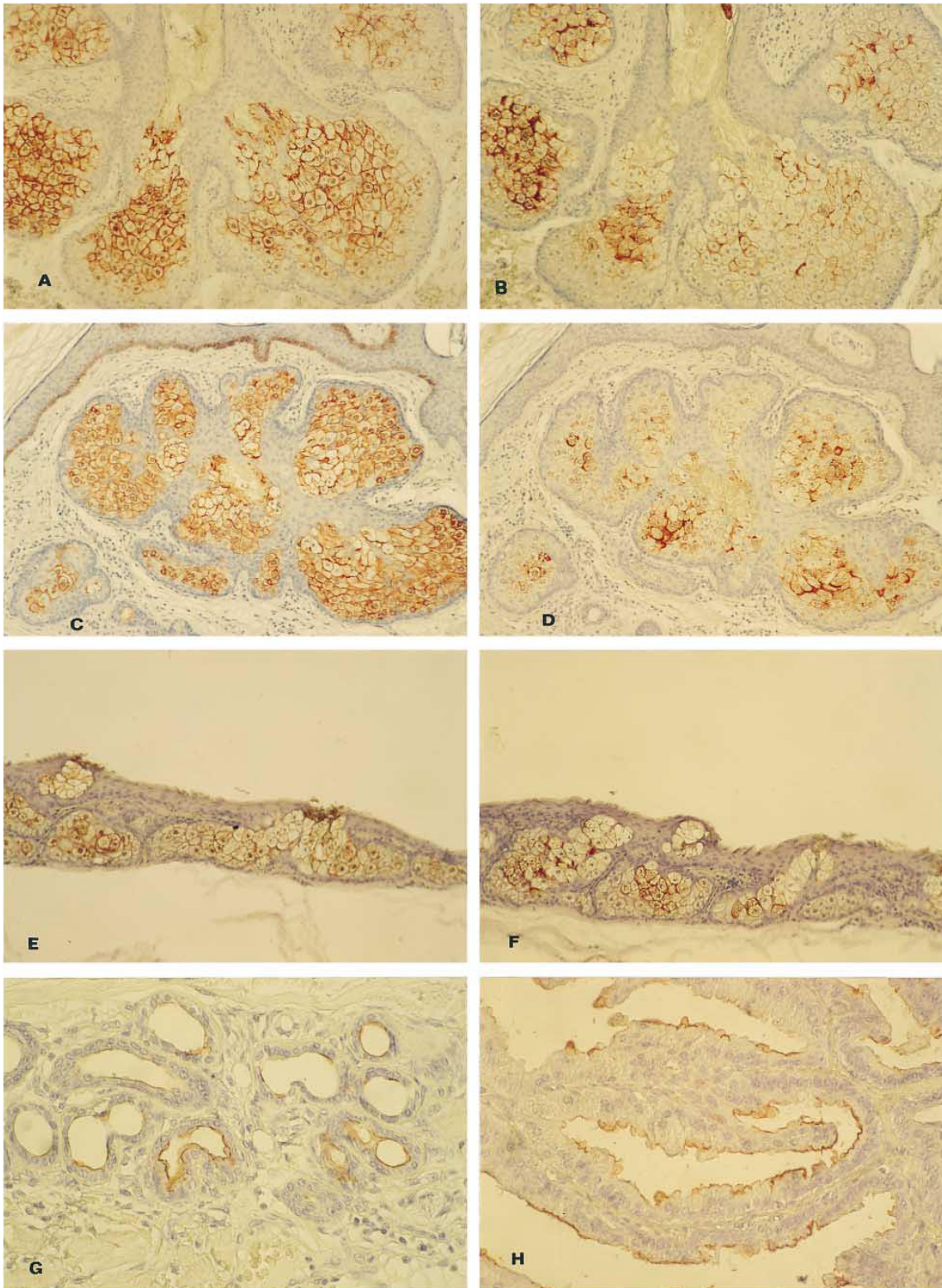
antibodies (1, 6). PNA cross-reacts to other  $\beta$ -galactosides, a fact that may explain why it labels several skin components, such as the surface of keratinocytes of the upper epidermal layers and of hair follicles (9), the epithelial component of hair follicle tumours (10) and a variable proportions of histiocytic cells in xanthogranulomas and Langerhans' cell histiocytoses (11). In the present study we never detected such reactivities either on normal or diseased skin. Therefore the results of studies with lectins are not comparable with those obtained with specific antibodies, at least in the specific case of T/Tn antigens.

Only few data exist up till now on the expression of T and Tn antigens detected with monoclonal antibodies in human skin (6, 7). We found consistent T and Tn expression within the cytoplasm of mature sebocytes but no expression on epidermal or hair-follicle keratinocytes. T antigen expression was stronger than the Tn, suggesting that in normal sebaceous glands the carbohydrate metabolism leads to glycosylation of the Tn antigen. Another explanation for this expression pattern is that the T antigen is progressively broken down into Tn antigen as sebocytes become more mature. Whatever the explanation, in keeping with these results we found strong expression of the Tn and mainly the T antigen in all congenital or acquired sebaceous proliferative lesions studied. In one case of sebaceous naevus we observed T antigen immunoreactivity within basal epidermal cells overlying the hyperplastic sebaceous glands. The significance of this finding is unclear; since this lesion is a hamartomatous condition involving epidermal adnexae and also the epidermis, it can be speculated that in this case some basal epidermal keratinocytes could have the potential to differentiate towards sebocytes. Alternatively, the hypothesis of a cross-reactivity with an unknown antigen cannot definitively be excluded.

On the other hand, infrequently we found T/Tn antigen immunoreactivity on normal sweat glands, and more frequently in some tumours arising therefrom, of both eccrine and apocrine origin. In these cases the staining was located over the apical pole of cells lining secretory or ductal lumina, and never showed a cytoplasmic pattern, as seen in sebocytes.

Up till now very few studies have addressed the issue of T and Tn antigen expression in the skin with the use of monoclonal antibodies. One study reported T and Tn antigen expression within fetal epidermis and epidermal appendages (7). In another work where five specimens of adult human skin were studied with monoclonal antibodies, it was reported that sebaceous glands expressed the T (but not Tn) antigen (6). The discrepancy between this study and our own, suggesting Tn antigen expression in sebaceous glands, is most likely due to the fact that different antibodies were used in these studies. Indeed, it has been shown that different anti-Tn antibodies do not produce the same staining patterns in various tissues, including the skin (6).

Whatever the explanation, a common finding of our study and the previous ones is the expression of the T antigen by sebocytes, suggesting that this molecule may serve as a good marker of sebaceous differentiation in human skin. Several antigens have been used for the immunohistochemical detection of sebocytes both *in vivo* and *in vitro*. Apart from PNA lectin (12) these include individual keratin polypeptides (13, 14), epithelial membrane antigen-EMA (15), biliary glycoprotein-BGP (16), OM-1 (17), BCA-225 (18) and lipase (19). The T antigen can be added to this list, and presents several



*Fig. 1.* (A) T antigen is strongly expressed within the cytoplasm of mature sebocytes; undifferentiated sebocytes and other cell types are negative. (B) Tn immunoreactivity is seen within the cytoplasm of the more differentiated sebocytes; basal and suprabasal sebocytes are unreactive (serial section with that of Fig. 1A). (C) Strong T-antigen immunoreactivity within sebaceous glands in naevus sebaceus. (D) Strong Tn-antigen immunoreactivity within sebaceous glands in naevus sebaceus (serial section with that of Fig. 1C). (E) T antigen expression in sebocytes present in the epithelial wall of a sebocystomatosis cyst. (F) Tn antigen expression in sebocytes present in the epithelial wall of a sebocystomatosis cyst (serial section with that of Fig. 1E). (G) Luminal expression of the T antigen in eccrine sweat glands. (H) T antigen immunoreactivity is seen on the luminal surface of cells in a case of hidradenoma papilliferum.

Table II. Expression of TF/Tn antigens in various tumours and dermatoses

Diagnosis	Total no.	T+	Tn+
Non-adnexal tumours	45	1	2
Seborrheic keratosis	6	0	0
Viral warts	3	0	0
Keratoacanthoma	6	0	0
Bowenoid papulosis	1	0	0
Clear-cell acanthoma	1	1/+	0
Warty dyskeratoma	1	0	0
Benign naevus	16	0	1*/±
Angioma	2	0	0
Granular-cell tumour	3	0	1/±
Dermatofibroma/scar	3	0	0
Xanthogranuloma/xanthelasma	2	0	0
Lipoma	1	0	0
Miscellaneous	12	2	0
Psoriasis	4	0	0
Dermatitis	2	1/+	0
Lichen planus	2	1/±	0
Grover disease/Pemphigus	2	0	0
Pseudolymphoma	1	0	0
Sarcoidosis	1	0	0

\* Spitz naevus.

advantages: it decorates (mature) sebocytes in a clear-cut pattern and can be detected on formalin-fixed, paraffin-embedded sections, thus allowing retrospective studies; it does not show cross-reactivity with the epidermis and hair follicle keratinocytes and tumours arising thereof, and, although not absolutely specific for sebaceous differentiation, it shows less cross-reactivity with sweat glands and their neoplasms as compared with EMA, the commonest sebaceous marker used in diagnostic dermatopathology; finally, it does not react (with insignificant exceptions) with non-epithelial cells and tumours. The significance of weak T antigen expression in mature epidermal keratinocytes in rare cases of inflammatory dermatoses (dermatitis, lichen planus) remains unknown; intriguingly, it is reminiscent of that concerning EMA, observed also (according to our experience much more frequently) in these conditions.

In conclusion, the T antigen appears to be a sensitive marker of sebaceous differentiation, useful for the study of adnexal cutaneous tumours. Its expression in cutaneous malignancies is currently under investigation.

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