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Toker cell related to the folliculo-sebaceous–apocrine unit: a study of horizontal sections of the nipple

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Abstract

Aims: Since Toker cell (TC) was first described in 1970, many interpretations concerning their origin have been offered in literature. We tried to investigate the histology of Toker cells in horizontal sections of the nipple. *Material and methods*: We have studied horizontal sections of the nipple in 10 cases from mastectomy specimens due to ductal carcinoma. We used conventional Hematoxylin–Eosin stains, as well as immunohistochemical staining for cytokeratin 7 in each case. Immunostaining for EMA, CEA and S-100 were also used in the three cases in which TCs were more numerous. In three cases, we made serial sections of the nipple. *Results*: We evidenced TC in 80% of our cases in the immunohistochemical study. The three cases that were studied with additional antibodies showed a phenotype CEA-, S-100-. While one of the cases was EMA+, the other two were EMA-. *Conclusions*: The location of TCs, together with the findings in the cases that were seriated, suggests a physical relation between TCs and the sebaceous glands. This latter hypothesis would also explain the immunophenotype of TCs that has been widely studied in literature, and partially corroborated by the current study.

Keywords: Toker cell, Paget disease, nipple, folliculo-sebaceous–apocrine unit, CK7.

Introduction

Toker cells (TCs) were described in 1970 by Cyril Toker [1]. He interpreted them as abortive ductular breast tubules. Although he performed serial sections of the examined specimens, the latter were more orientated to the search of a subjacent carcinoma, and no connection with underlying ductular tubules was described. He also described that TC were mainly located at the summit of the nipple.

All this led us to design a model to investigate the evidence of TC in nipples with horizontal sections, focusing on the summit, in order to find any new details that could give any clues concerning the origin of TC.

Material and methods

We obtained horizontal sections from the nipples of mastectomies that had been performed due to infiltrating breast carcinoma. None of the nipples were involved by the carcinoma in the studies with Hematoxylin–Eosin, as a rule. Moreover, the distance between the tumor and the areolar margin was at least of 2 cm.

Two horizontal sections of the nipple were included in each paraffin block, as shown in Figure 1. One of the sections always corresponded to the tip of the nipple (Figure 1D).

Routine slides, stained with Hematoxylin–Eosin (HE), were obtained from each of the cases. We also performed an immunohistochemical study with antibody for cytokeratin (CK) 7 in each case (DAKO, mouse anti-human antibody, Clone OV–TL, ready-to-use, code N1626).

We evaluated the presence of Toker cells in the HE sections, as well as in the immunohistochemical staining. In three cases (numbers 1, 2 and 4), seriated sections of the nipple were immunostained with CK7 antibody, in order to investigate the connections of the TC population.

In three cases (1, 4 and 10), we performed an additional immunohistochemical study with the following antibodies: carcinoembryogenic antigen (CEA) (DAKO, mouse anti-human antibody, Clone II–7, ready-to-use, code N1586), S-100 protein (DAKO, rabbit anti-cow antibody, ready-to-use, code N1573), and epithelial membrane antigen (EMA) (DAKO, mouse anti-human antibody, Clone E29, ready-to-use, code N1504).

The reason why those three cases were chosen was that TCs were easily found in them (as shown below in the results), so the evaluation of the rest of the markers would be more reliable.

Results

All patients were female. The clinical details about the patients are shown in Table 1.

Table 1 – ***Details about the patients included in the study***

Case	Age [years]	Breast	Diagnosis	TC: HE	CK7	S-100	CEA	EMA
1.	70	Right	IDC	Yes	More than occasional intraepidermal cells; intense cytoplasmic expression	Negative	Negative	Negative
2.	50	Left	IDC	No	Occasional intraepidermal cells; intense cytoplasmic expression	ND	ND	ND
3.	57	Left	IDC	No	No intraepidermal cells	ND	ND	ND
4.	38	Right	Infiltrating tubulo-lobular carcinoma	Yes	More than occasional intraepidermal cells; intense cytoplasmic expression	Negative	Negative	Intense cytoplasmic expression
5.	72	Right	IDC	No	Occasional intraepidermal cells; intense cytoplasmic expression	ND	ND	ND
6.	68	Left	IDC	No	Occasional intraepidermal cells; intense cytoplasmic expression	ND	ND	ND
7.	73	Right	IDC	No	No intraepidermal cells	ND	ND	ND
8.	76	Left	IDC	No	Occasional intraepidermal cells; intense cytoplasmic expression	ND	ND	ND
9.	77	Left	IDC	No	Occasional intraepidermal cells; intense cytoplasmic expression	ND	ND	ND
10.	49	Left	IDC	Yes	More than occasional intraepidermal cells; intense cytoplasmic expression	Negative	Negative	Intense cytoplasmic expression

IDC: Infiltrating Ductal Carcinoma; HE: Hematoxylin–Eosin; ND: not done; CK7: Cytokeratin 7; CEA: Carcinoembryonic Antigen; EMA: Epithelial Membrane Antigen.

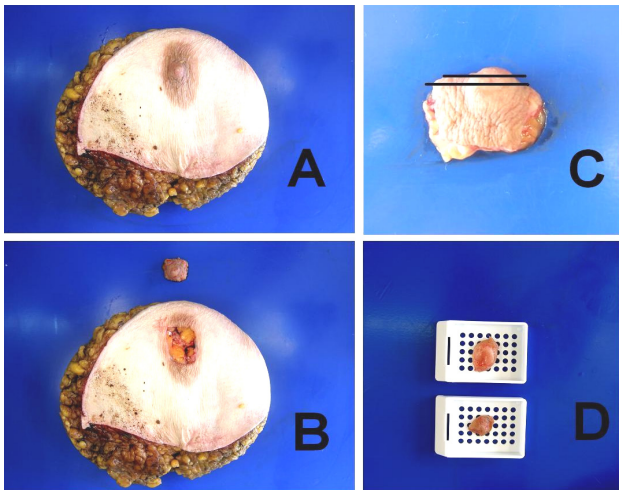

Figure 1 – ***The image shows how the nipples of the specimens were processed. From the mastectomy specimens (1A), the nipple was sectioned (B), and horizontally sectioned (C). That way, two blocks were obtained from each nipple: one from the tip and one from the base (D)***

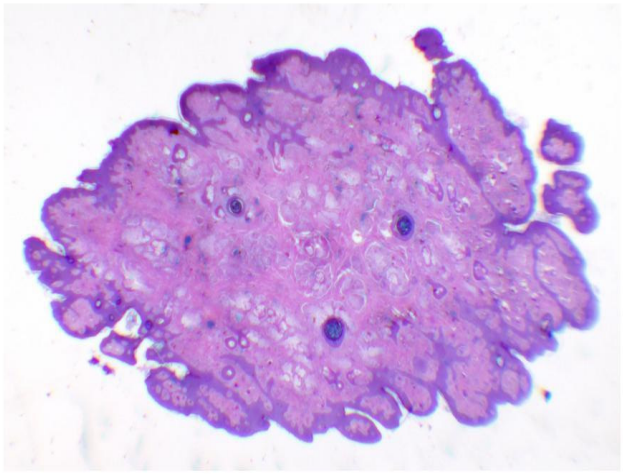

Figure 2 – ***Low power view of the section corresponding to the tip of one of the cases, showing many sebaceous glands. The picture is somewhat blurred, due to the very low magnification needed to show the whole nipple***

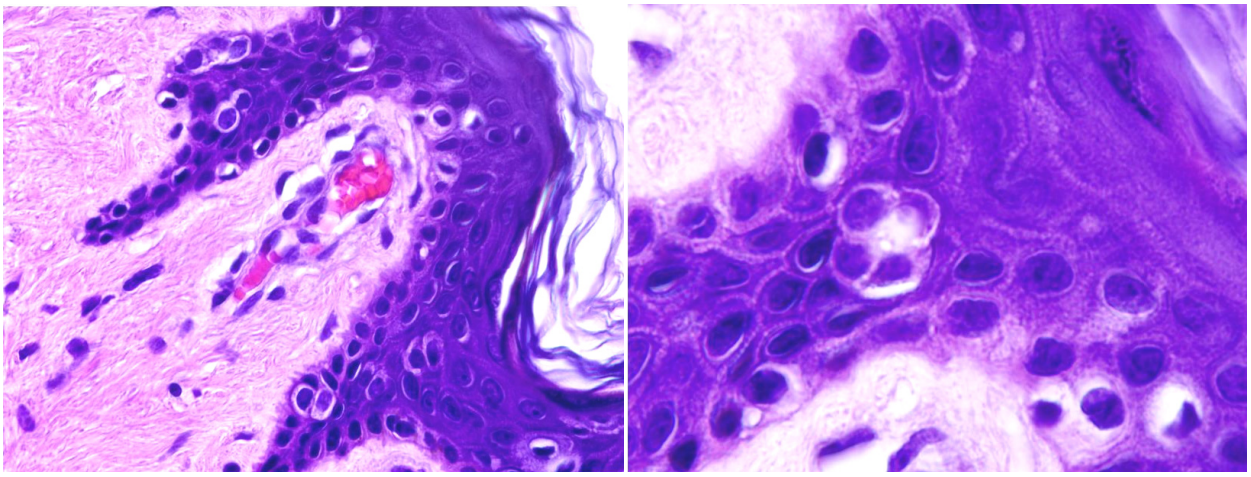

Figure 3 – ***Toker cells were easily evidenced in 30% of the cases, either as scattered cells (left), or as glandular structures (right)***

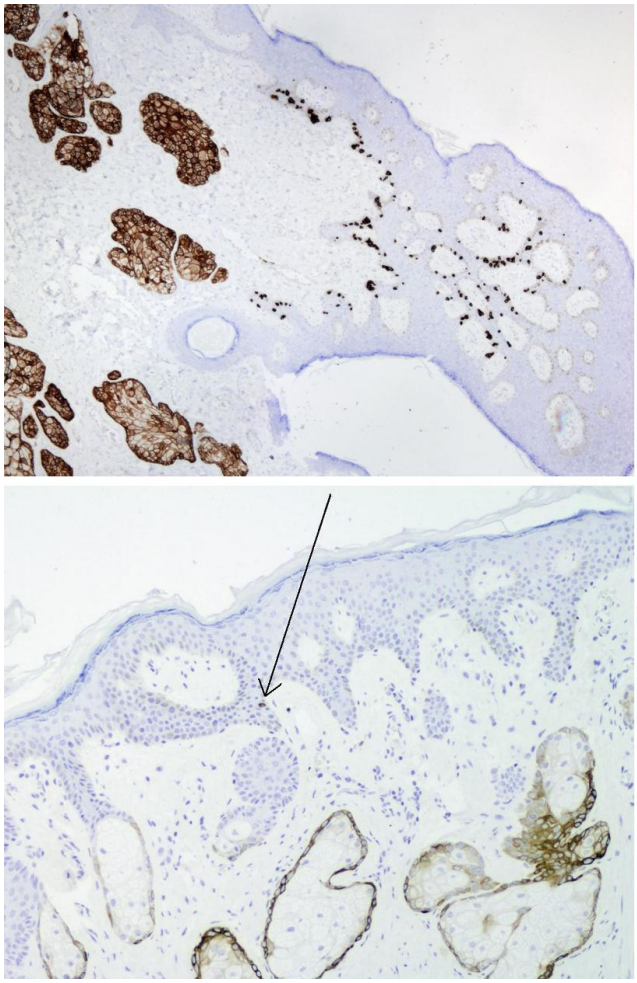

Figure 4 – ***Toker cells, evidenced by immunostaining with antibody for CK7. Although in some cases, TCs were scattered (top), in others they were abundant (bottom)***

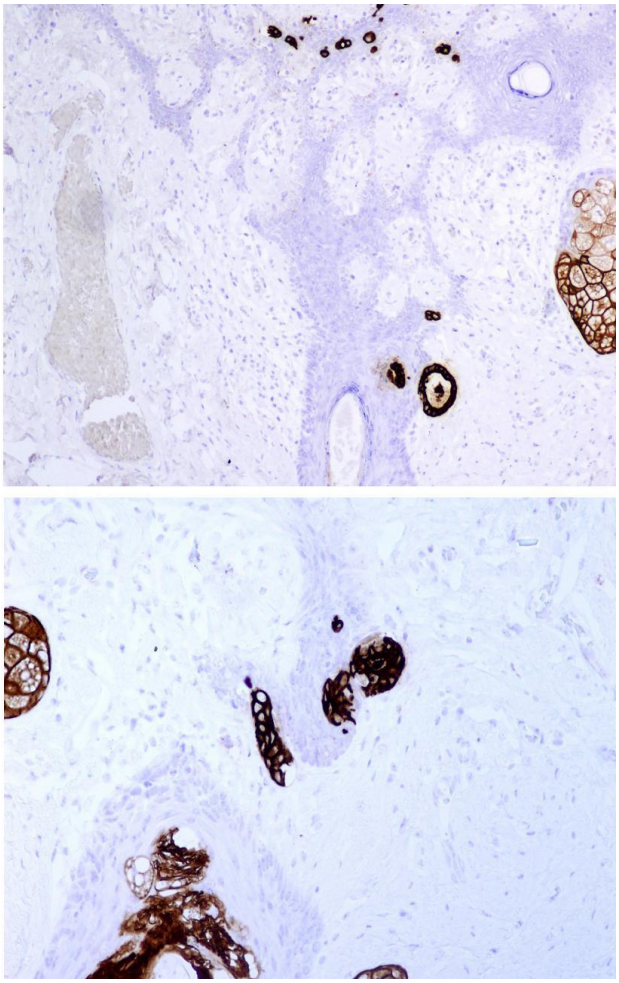

Figure 5 – ***Seriated sections from the cases showed that some scattered cells were located intraepidermally above the sebaceous gland, but we could not prove continuity with the cells that were located higher in the epidermis***

The slide, corresponding to the tip of the nipple, showed abundant sebaceous glands with ductular breast tubules (Figure 2). In none of the cases, tumoral infiltration of the nipple was evidenced, in either the stroma or lymphatics, either with the routine study or with the immunohistochemical study.

We found TCs in three of the nipples, in the routine study with HE (30%) (Figure 3), which sometimes formed glandular arrangements with a central lumen (Figure 3, right). On the contrary, we evidenced TCs in eight cases (80%) in the immunohistochemical study. They varied in quantity from many cells to just scattered cells (Figure 4). TCs were mainly evidenced in the epidermis above the sebaceous glands (Figure 4, bottom).

In three cases (numbers 1, 2 and 4), we obtained seriated sections immunostained with CK7 antibody. Some individual cells were detached into the epidermis from the sebaceous duct (Figure 5), but we could not prove the connection between the gland and the TC population. All three cases that were investigated for S-100 and CEA by TCs showed a lack of expression of these markers by TCs. The latter, nevertheless, expressed EMA in two of the cases, and failed to express it in the other case.

Discussion

Toker cells (TCs) were described in 1970 by Cyril Toker [1]. Nevertheless, as the author admitted, there is a previous reference in literature regarding these cells in normal nipples [2], although they were misinterpreted as melanocytes.

In his original study, Toker did not describe if he used horizontal or vertical sections of the nipple. In the photographs presented by him, clear intraepithelial cells were prominent, numerous and beautifully shown. Nevertheless, in his studies from nipples from autopsies, they managed to find TCs in only 12% of the cases, which showed that TCs were not so easily found. Yao DX *et al.* greatly improved that percentage by detecting Toker cells with immunostaining for CK 7, in up to 44% of their cases [3]. Although they do not specify if they used transversal or perpendicular sections of the nipple, the latter seems the most probable, according to the pictures shown in the report. Our current results using horizontal sections improved the previous studies, since TCs were found in 80% of the cases with immunohistochemistry.

Toker presented tubular intraepithelial images made of cells with similar morphologic characters as the

single cells, which made him hypothesize a glandular origin for TCs, although he clearly claimed that they "were clearly not of lactiferous, sebaceous, or sudoriferous origin" [1]. Instead, he suggested that they were abortive ductular tubules, because they were made of only one cell layer. However, he admitted that they saw "no possibility of resolving the question as to which element preceded".

Although they obtained many sections, they did not prove continuity with ductular breast tubules from underneath, hypothesizing either a migration of the tubules from the breast or an abortive mammary differentiation within the basal layer. Most modern studies, which have used immunohistochemistry, do not mention a direct connection between TC and the ductular tubules [3].

Toker saw these cells mainly along the summit of the nipple. Others have also described this latter topography of TCs, which seems to be more numerous around the opening of the lactiferous duct, and decreases in number the further away from the summit [3]. Still others have suggested that TCs are germinative or primitive cells of the lactiferous duct [4]. Other additional interpretations of TCs are as abortive mammary ductular cells [3].

We investigated the nipple in horizontal sections. The nipple has around 12 to 20 orifices through which the milk ducts drainage [5]. The number of milk ducts that were investigated increased in a horizontal section in comparison to a vertical one due to the same reasons that have been argued for the horizontal sections of hair follicles when studying alopecias [6, 7].

Since the main number of TCs is located at the summit of the nipple, this is the part of the nipple that should be horizontally investigated. Although, the horizontal section has been suggested before some authors emphasized on the base of the nipple as a complementary study to the vertical section of the summit [8]. A horizontal sectioning approach to the study of the nipple was presented in literature for the study of the mammary ducts [9].

Our results suggest that TCs are physically related to the sebaceous gland. One of the reasons, why we think that this relation could be seen so clearly in our study, is that the sebaceous glands abut the epidermal nipple, in a nearly perpendicular way to the main axis of the nipple. Since we had cut the nipple horizontally, we could easily follow the secretory duct of the sebaceous gland, a difficult task in vertical sections, where the continuity between these glands and the TCs population would have been missed after some sections.

One wonders why TCs are only evidenced in certain areas of the body, where PD is also common, and not in all areas which are rich in sebaceous glands. It should be remembered, nevertheless, that the sebaceous gland opens at the nipple independently of hair follicles, which would favor the intraepidermal visualization of scattered cells related to the gland.

Our findings would also explain why the immunophenotype expressed by TCs (CEA-, CK7+, S-100-) is shared by the sebaceous gland [10] (this is also the pattern that we observed in our three cases that were investigated for CEA and S-100). CK7 is strongly expressed by the secretory part of the sebaceous gland, as well as by the sebaceous duct, as can be seen in some of the figures presented in our report. This would explain why any intraepidermal part of the sebaceous gland (the high secretory part of the gland or already the duct) would also express CK7. Nevertheless, while the secretory part of the sebaceous gland is EMA+ [10], the duct does not express EMA [11]. This would also explain the immunohistochemical findings regarding TC and EMA: TC hardly ever express EMA (the same as the duct of sebaceous glands), but they can occasionally express it [12–14] (which would be in consonance with a sebaceous origin, if admitting that the high area of the secretory part of the sebaceous gland can occasionally be located inside the epidermis). From the three cases that we investigated for EMA immunoexpression, only in one of them, TCs failed to express the marker, while the other two cases were positive.

Nevertheless, we think that the close association between TCs and the sebaceous gland, observed in this study, does not necessarily mean a "sebaceous differentiation" of TCs. In the latter years, the concept of the folliculo-sebaceous–apocrine unit as a source of common differentiation has been recognized in literature [15, 16], and so, it would be more correct to speak of a relation between TC and that unit.

It is interesting how another group, following a totally different approach, reached similar conclusions to ours when studying the extramammary PD: in 2005, Regauer S found that the population of extramammary PD was "reminiscent of sebaceous glands with mature sebocytes and germinative keratinocytes" [17]. She interpreted that the disease might be "a proliferation of adnexal stem cells residing in the infundibulo-sebaceous unit of hair follicles and adnexal structures" [17]. In the beautiful images of her report, one can see several examples of PD cells associated to sebaceous glands. Nevertheless, it is our belief that the population that we identified associated to the sebaceous gland belonged to what has been known in history as TCs, and not necessarily part of the mammary PD. The large distance, between the tumor and the nipple, which we set down as a requirement to select our cases, would be sort of a guarantee for such an assertion.

Conclusions

The location of TCs, together with the findings in the cases that were seriated, suggests a physical relation between TCs and the sebaceous glands. This latter hypothesis would also explain the immunophenotype of TCs that has been widely studied in literature, and partially corroborated by the current study.

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