Annals of Diagnostic Pathology 20 (2016) 44–47

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

Distinctive immunostaining of claudin-4 in spiradenomas☆,☆☆

Nuri Yiğit, MD [a,*], Ertuğrul Çelik, MD [b], İbrahim Yavan, MD [a], Armağan Günal, MD [a], Bülent Kurt, MD [a], Yıldırım Karslıoğlu, MD [a], Önder Öngürü, MD [a], Ayhan Özcan, MD [a]

[a] Department of Pathology, Gülhane Military Medical Academy and School of Medicine, Ankara, Turkey
[b] Department of Pathology, İzmir Military Hospital, İzmir, Turkey

ARTICLE INFO

Keywords:
Claudin-4
Spiradenoma
Intercellular bridge proteins
Eccrine tumors
Skin tumors

ABSTRACT

The intercellular bridges are essential structures in maintaining the histologic organization of the epithelium, while providing a very efficient way to exchange molecules between cells and transduction of the cell-to-cell and matrix-to-cell signals. Derangement in those important structures' physical integrity and/or function, which can be assessed by the presence or absence of several intercellular bridge proteins including claudin-4, E-cadherin, and β-catenin, was found to be related to several phenomena in the path to the neoplastic transformation. However, these proteins have not been studied in the wide variety of the skin neoplasms, in detail. Herein, we immunohistochemically assessed the expression patterns of these 3 intercellular bridge proteins on a total of 86 epidermal and eccrine adnexal tumors including basal cell carcinoma, squamous cell carcinoma, poroma, spiradenoma, syringoma, and hidradenoma. We observed a selective and distinct claudin-4 expression in the ductal-type cells of all cases of spiradenomas. Similarly, in the poromas, syringomas, and hidradenomas, claudin-4 was only positive in the luminal cells of microcystic structures, although not as conspicuous as in the spiradenomas. On the other hand, E-cadherin and β-catenin were positive in almost all types of the tumors, in a way which was not contributory to differentiate from each other. In conclusion, we think that claudin-4 can be helpful at least in making a reliable differential diagnosis of spiradenoma when overlapping morphologic features do not allow to further subclassification in the overwhelming variety of the adnexal tumors.

1. Introduction

Normal epithelia maintain their intricate and vulnerable organizations mostly by intercellular bridges which perpetuate the arrangement of the cells in spatial order as well as the transportation of crucial molecules between the adjacent cells. Intercellular bridges, which comprise the highly modified portions of the neighboring cells' membranes, are complex structures harboring some important proteins which play crucial roles in the functions of desmosomes, gap junctions, zonula adherens, and tight junctions [1,2]. The claudins, occludins, cadherins, and catenins are some of the well-known constituents of the membrane and membrane-associated cytoskeletal proteins at the site of intercellular bridges [3,4].

Up to date, in many tumors, dysregulation of those junctional proteins has been shown that they might have some promoter effects throughout the event in the tumorigenesis [5]. Overexpression or underexpression of such molecules was found to be resulted in loss of cell-cell cohesion, increase in motility, invasiveness, and metastasis [6,7].

Despite the expanding resources and the ancillary techniques, diagnosis of the most skin tumors is still based on the morphologic findings and pattern. Only few cases having overlapping histologic features cause diagnostic difficulties and warrant to be studied with further workups. In routine pathology practice, the immunohistochemical markers frequently provide useful data to make correct diagnosis. However, the contribution of immunohistochemistry in differential diagnosis has been shown limited for many skin neoplasms in contrast to the nonskin tumors. Hence, this inability led to the requirement of finding further ancillary techniques when needed.

Herein, we investigated immunohistochemical characteristics of 3 intercellular bridge proteins including claudin-4, E-cadherin, and β-catenin on a total of 86 cases of different types of frequently encountered malignant and benign skin tumors, to analyze their expression patterns.

2. Materials and methods

2.1. Case selection

We retrospectively selected 86 cases in total, including 20 basal cell carcinomas (BCCs), 20 squamous cell carcinomas (SCCs), 20 poromas, 10 spiradenomas, 10 syringomas, and 6 hidradenomas among the files of the Department of Pathology of Gulhane Military Medical Academy

☆ Conflict of interest statement: The authors declare no conflict of interest.
☆☆ Disclosure statement: The authors declare no relevant financial or nonfinancial relationship to disclose.

* Corresponding author at: Department of Pathology, Gülhane Military Medical Academy and School of Medicine, Ankara 06010, Turkey. Tel.: +90 312 304 3734; fax: +90 312 304 3700.
E-mail addresses: nyigit@gata.edu.tr (N. Yiğit), ertugrul_celik@hotmail.com (E. Çelik), ibrahimyavan@gmail.com (İ. Yavan), agunal@gata.edu.tr (A. Günal), bkurt@gata.edu.tr (B. Kurt), ykarslioglu@gata.edu.tr (Y. Karslıoğlu), oonguru@gata.edu.tr (Ö. Öngürü), aozcan@gata.edu.tr (A. Özcan).

http://dx.doi.org/10.1016/j.anndiagpath.2015.10.005
1092-9134/

in Ankara. All of them were excisional biopsy specimens. Archival slides of all cases were reevaluated to confirm their diagnosis. Five uninvolved normal skin samples were also used as a control group in the study. The specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin, and processed routinely, and the sections were stained with hematoxylin and eosin (H&E). The design of this study was approved by the local ethical committee of Gulhane Military Medical Academy in Ankara.

2.2. Immunohistochemistry

All tissue sections were immunostained against claudin-4 (rabbit polyclonal, 1:150; Neomarkers, Fremont, CA), E-cadherin (rabbit monoclonal, 1:200; Cell Marque, Rocklin, CA), and β-catenin (mouse monoclonal, 1:200; Dako, Carpinteria, CA) by using an autostainer (Ventana Benchmark XT; Ventana Medical Systems, Inc, Tucson, Arizona), following the manufacturer's protocol. Appropriate staining was confirmed using external positive controls for each antibody. The intensity of the staining was scored as follows: 0, negative; +, weak; ++, moderate; or +++, strong. All stained slides have independently been evaluated by 2 blinded pathologists (N.Y. and E.Ç.).

3. Results

Claudin-4 exhibited membranous staining without any cytoplasmic or nuclear marking in all groups. In the control group, claudin-4 exclusively and faintly highlighted upper layer of epidermis as was documented previously (Fig. 1a) [8]. The inner root sheath of hair follicle and some attached sebaceous cells showed weak membranous labeling (Fig. 1b). Also, both secretory and ductal cells of the eccrine sweat glands demonstrated strong and membranous marking (Fig. 1c). Interestingly, in all spiradenomas, claudin-4 displayed strong and membranous staining in the cells, which were morphologically corresponded to the ductal structures on H&E-stained slides, and spared the other components of the glandular cells of small dark and larger pale cells (Fig. 1d-f). Appearance of this distinct ductal staining on the sections gave the impression of an easily detectable lace-like network, which exposed the typical ductal part of the spiradenomas. Subsequently, to be sure whether these stained cells in spiradenomas are ductal nature indeed, we used further immunohistochemical stain against carcinoembryonic antigen (CEA) (rabbit polyclonal, 1:400, Cell Marque, Rocklin, CA) labeling of which was reported to be more specific for ducts than for the secretory portion, on a few selected cases of the spiradenomas [9]. We observed moderate staining in the luminal surface of the many ductal cells with a minute population of secretory cells, confirming that the claudin-4 selectively highlighted the ductal parts of the spiradenomas (Fig. 1g). When we compared the immunostaining intensity of claudin-4 and CEA in these ductal cells, claudin-4 immunoexpression was definitely stronger and extensive than CEA.

On the other hand, claudin-4 was somewhat positive in the luminal cell layer of microcysts in the poromas, syringomas, and hidradenomas, in a way of noncontributory to their differential diagnosis (Fig. 2a-c).

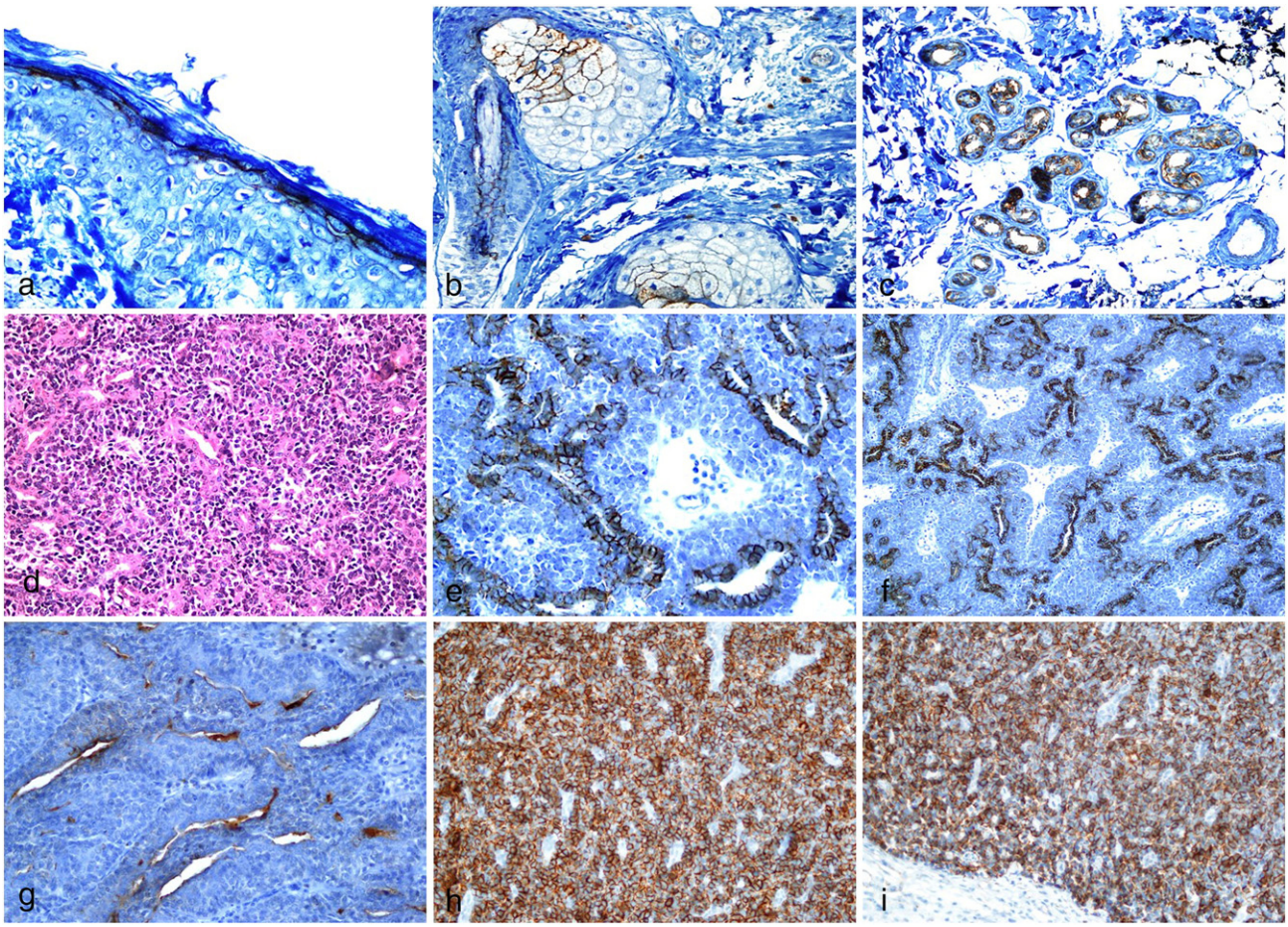

Fig. 1. In the uninvolved skin sample, claudin-4 immunostaining was weak in the granular layer of epidermis (a), moderate in the cells of inner root sheath of hair follicle with expression in some sebaceous cells (b), and strong in the eccrine sweat glands (c) (immunoperoxidase, original magnification ×400). (d) Spiradenoma containing the small dark and larger pale cells with scattered ductal structures (H&E, ×100). (e and f) Claudin-4 expression in the tumor selectively reveals the ductal component, with a lace-like appearance, on a background of the purely negative glandular cells (immunoperoxidase, ×400 and ×200, respectively). (g) CEA highlighted the luminal surfaces of some ductal cells with a weaker intensity compared with the claudin-4 (immunoperoxidase, ×400). (h and i) E-cadherin and β-catenin showing diffuse and strong membranous staining in all cell types of spiradenomas (immunoperoxidase, both ×400).