Cylindroma as Tumor of Hair Follicle Origin

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TO THE EDITOR

Cylindromas are usually benign skin appendage tumors that are most frequently found in scalp and neck skin, with a strong predilection for middle-aged and elderly females (Weedon, 2002). Cylindromas are characterized by irregularly shaped islands of basaloid cells arranged in a ''jigsaw puzzle'' pattern, which are surrounded by an eosinophilic hyaline sheath (Figure 1a, arrowheads). They exhibit multiple molecular defects in their basement membrane zone, including the lack of mature hemidesmosomes, defective processing of laminin 5 (Tunggal *et al.*, 2002), and abnormalities in collagen IV alpha 1- and alpha 5-chain expression (Quatresooz and Pierard, 2005). In the tumor islands, a peripheral palisade of relatively undifferentiated cells with small, dark nuclei (Figure 1b, arrows) can be distinguished from more differentiated cells with large, pale nuclei, which resemble ductal or secretory cells (Requena *et al.*, 1998; Weedon, 2002; Klein *et al.*, 2005; Figure 1b, **).

Conventional wisdom classifies cylindroma as a neoplasm of apocrine differentiation (Weedon, 2002; Klein *et al.*, 2005), even though circumstantial ultrastructural and immunohistochemical evidence has also been presented in favor of an eccrine origin of cylindroma (Crain and Helwig 1961; Cotton and Braye, 1984; Penneys and Kaiser, 1993; Ishihara *et al.*, 1998). However, the fact that cylindromas exclusively arise in hair follicle-bearing regions of the integument, but not in palmoplantar skin – which is rich in eccrine glands, yet devoid of pilosebaceous units and apocrine glands – already argues against an eccrine origin. Despite the exploitation of a wide range of enzymehistochemical, immunohistological, and ultrastructural markers, none of these has as yet been generally accepted as definitive proof for one or the other concept, and the histogenesis of cylindromas has remained a subject of intense and controversial debate (for discussion, see Requena *et al.*, 1998; Weedon, 2002; Klein *et al.*, 2005).

This debate is important as it directly relates to the question of whether cylindromas arise from pluripotent hair follicle epithelial stem cells (as speculated, e.g., by Schirren *et al.* (1995) and Requena *et al.* (1998)) that can differentiate into the follicular, apocrine, or sebaceous lineage, but do not generate eccrine sweat glands, or whether they arise from a distinct epithelial precursor population that would give rise only to eccrine glands, but to none of the differentiation pathways recognized for the progeny of hair follicle epithelial stem cells (Fuchs *et al.*, 2004).

During fetal hair follicle morphogenesis, in human skin, the apocrine gland arises from the keratinocytes of the developing outer root sheath. These, in turn, arise from epithelial stem cells located in the bulge region of the outer root sheath. The same is true of the sebaceous gland (Philpott and Paus, 1998; Requena *et al.*, 1998). Therefore, the concept of an apocrine histogenesis, and even the sebaceous-type differentiation pattern that one can sometimes find in a cylindroma (Requena et al., 1998), are fully compatible with that of a hair follicle origin of cylindroma: follicular, sebaceous, and apocrine differentiation pathways all arise from the same precursor cells in the hair follicle outer root sheath, likely from bulge epithelial stem cells or their immediate progeny.

Even the previously postulated eccrine origin of cylindroma (Crain and Helwig 1961; Cotton and Braye, 1984; Penneys and Kaiser, 1993; Ishihara *et al.*, 1998) would not be entirely irreconcilable with a follicular histogenesis of cylindroma, if one considers the recognized pluripotentiality of hair follicle epithelial stem cells: these can regenerate a complete epidermis (Taylor *et al.*, 2000; Fuchs *et al.*, 2004; Morris *et al.*, 2004; Wu *et al.*, 2004), from where eccrine glands originate (Hashimoto *et al.*, 1966).

As none of the previously examined markers has been universally accepted as definitive proof for either an apocrine or eccrine origin of cylindroma (Weedon, 2002, Klein *et al.*, 2005), we argued that the question of whether or not cylindroma originates from epithelial cells committed to the *follicular–apocrine–sebaceous* or to the *eccrine* differentiation pathway should be settled, if one can demonstrate the expression of markers of advanced hair follicle-type differentiation that are specific to the hair follicle and that are definitely not expressed in eccrine glands.

Therefore, we immunostained cylindroma cryosections obtained during routine surgery from ''turban tumors'' of three female (60, 65, and 69 years old) patients with Brooke–Spiegler syndrome with informed consent (the medical ethical committee of the Max-Planck-Institute for Biochemistry approved all the studies described and the study was conducted according to the Declaration of Helsinki Principles), with specific antisera against human keratin 17 (K17), human hair keratin hHb2 (Langbein *et al.*, 2001), human K6hf (Winter *et al.*, 1998), or human K6irs1 (Langbein *et al.*, 2002). Although K17 expression is not entirely specific to human hair follicles, in normal human skin, it is most prominently expressed in the hair follicle outer root sheath, and is absent from eccrine glands and their ducts. Human hair keratin hHb2 is selectively expressed in the hair cuticle, K6Hf in the companion layer, and K6irs1 in the Huxley, Henle layers, and the cuticle of the inner root sheath (Winter *et al.*, 1998; Langbein *et al.*, 2001, 2002).

Horizontal sections of scalp hair follicles immediately adjacent to the

Abbreviation: K17, keratin 17

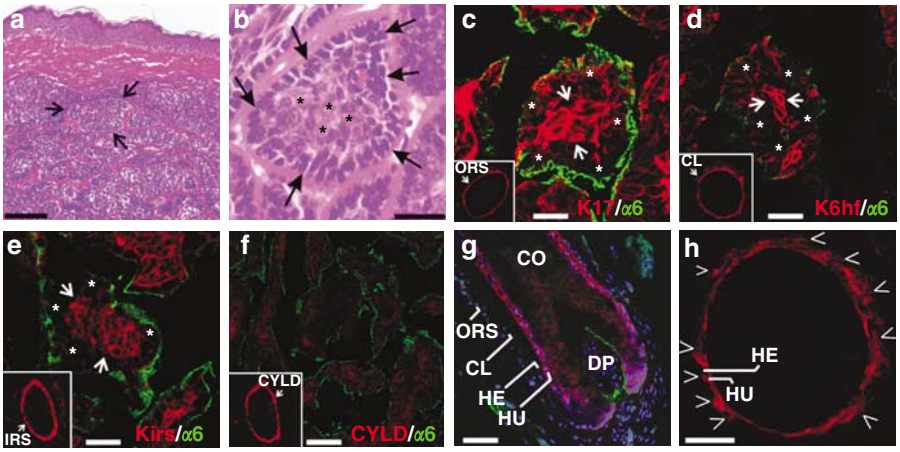

Figure 1. Expression of human hair follicle keratins and CYLD in cylindroma. Periodic acid Schiff (PAS) stain (LM) of cylindroma derived from female patient with familial cylindromatosis (Brooke–Spiegler syndrome) and multiple ''turban tumors'' (**a** and **b**; bars 100, respective 25 μm). Undifferentiated cells with small, dark nuclei (**b**, arrows) can be distinguished from more differentiated cells with large, pale nuclei (**b**, **). For immunohistochemistry and immunofluorescence, skin samples or cylindroma sections were frozen in 22-oxacalcitriol (OCT; Thermo Shandon) and cryosections of 10 μm thickness were cut and fixed with methanol/acetone. After blocking in 3% BSA/phosphate-buffered saline, the sections were stained with the primary antibodies directed against K17 (1:4000; **c**), K6irs1 (1:1:500; **d**), K6Hf (1:2000; **e**), and CYLD (1:500; **f**–**h**). The basement membrane was demarcated with α6-integrin antibodies (**c**–**f**). After washing, the sections were stained with specific secondary antibodies and DAPI (4′,6-diamidine-2′-phenylindole dihydrochloride) (**g**) before mounting and analyzing with Leica confocal microscope. The arrows in (**c**)–(**e**) represent less differentiated tumor epithelium, whereas the asterisks represent more differentiated layers of the tumor epithelium (bars = 25 μm). The insets in (**c**)–(**f**) shows expression of scalp hair follicle keratins immediately adjacent to the cylindroma mass. CYLD expression pattern (Huxley layer; HU) in normal human scalp hair follicles (**g**) and arrowheads in (**h**) represent expression pattern of CYLD in Huxley cells (''Flügelzellen'') (bars = 25 μm).

cylindroma mass were employed as internal positive control and showed the expected immunoreactivity patterns (Figure 1c–e, insets). The tumors were diagnosed as a typical benign cylindroma by pattern analysis of the classical diagnostic criteria established for this tumor (Figure 1a and b; Requena *et al.*, 1998).

As shown in Figure 1, cylindroma sections displayed prominent cytoplasmatic immunoreactivity of the tumor epithelium for K17, K6irs1, K6Hf (Figure 1c–e), and hHb2 (not shown). The observed expression of K17 in cylindromas (which has previously been noted in trichoepithelioma, but not cylindroma; Schirren *et al.*, 1995) is also consistent with a hair follicle-type differentiation of this tumor. The expression of hair-specific keratins suggests that the examined tumors could not possibly be of eccrine origin. Even though this remains to be verified by the analysis of additional cylindroma specimen from different individuals, given the very typical morphology of the examined cylindroma (Figure 1a and b), our data imply that this tumor generally is of hair follicle origin.

For K17, K6irs1, and K6Hf, a differential expression pattern was noted: a weaker basal layer immunoreactivity (corresponding to the peripheral, palisading layer of less differentiated tumor epithelium; Figure 1c–e, **) contrasted with a stronger immunoreactivity in the more differentiated layers of the tumor epithelium (Figure 1c–e, arrowheads). This is consistent with the concept that the neoplastic transformation giving rise to cylindroma development occurs in transit amplifying cells derived from a precursor cell pool of outer root sheath keratinocytes that are already committed to the hair follicle-type differentiation pathway, but are still capable of differentiating into various, distinct phenotypes within the follicular–apocrine–sebaceous spectrum of epithelial differentiation. As hair follicle epithelial stem cells can also enter into the epidermal pathway of epithelial differentiation (Taylor *et al.*, 2000; Fuchs *et al.*, 2004; Wu *et al.*, 2004), from where, in theory, even an eccrine-gland-type differentiation pattern could then develop, even ''eccrine'' features of a cylindroma are not necessarily incompatible with a primary follicular origin of this neoplasm.

A truly convincing, specific marker for *human* epithelial *hair follicle* stem cells is still not available (with even the specificity of K15 expression as such a marker still being disputed; cf. Li *et al.* (1998), Porter *et al.* (2000), and Morris *et al.* (2004)), and label-retaining experiments can evidently not be performed in cylindroma patients. Therefore, the presence and location of epithelial hair follicle stem cells and/or their (still highly undifferentiated, yet already partially committed) progeny within the cylindroma epithelium remain to be demonstrated.

The examined patients suffered from familial cylindromatosis (Brooke–Spiegler syndrome; OMIM numbers 123850, 132700, 313100, and 605041). The gene for familial cylindromatosis was mapped to chromosome 16q12–q13 by linkage analysis (Biggs *et al.*, 1995). Subsequently, the *CYLD* gene was discovered by positional cloning, and mutations in *CYLD* – a tumor suppressor gene – were identified in families with this disease (Bignell *et al.*, 2000; Poblete-Gutierrez *et al.*, 2002). These mutations predict truncation or absence of the encoded protein (Bignell *et al.*, 2000). In affected patients, cylindromas may arise through constitutive NF-κB activation (e.g. owing to the inability of mutated *CYLD* to downregulate this activation; Brummelkamp *et al.*, 2003; Regamey *et al.*, 2003).

Therefore, we also employed a specific rabbit antiserum we have most recently raised against a consensus sequence of mouse and human CYLD (Massoumi R, Chmielarska K, Hennecke K, Pfeifer A, Fässler R (2006) Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-κB signaling). Figure 1g and h shows the first evidence that CYLD protein is prominently expressed in the inner root sheath of normal human scalp hair follicles (Huxley layer; HU), including

in ''Flügelzellen'' (Figure 1h, arrow heads).

We also demonstrate here that CYLD immunoreactivity is dramatically downregulated in the epithelium of familial cylindromas (Figure 1f), compared to its strong immunoreactivity in a defined compartment of the hair follicle epithelium of the same patient (Figure 1f, inset). Even though this downregulation of CYLD immunoreactivity would seem to be consistent with a role for defective or insufficiently produced CYLD protein in cylindroma development, the current study does yet allow to draw any conclusions on the functional role of CYLD in the pathogenesis of familial cylindroma. However, the fact that this tumor suppressor gene product, which shows its expression maximum in human hair follicle inner root sheath keratinocytes (Figure 1f (inset), g, and f), is expressed at all in a cylindroma further supports the concept that this skin appendage tumor is of follicular origin.

In summary, our data suggest that cylindroma epithelium likely originates from epithelial hair follicle, whose exact differentiation pathway (follicular *versus* apocrine *versus* sebaceous, and may be even eccrine) may be dictated by the nature of the epithelial–mesenchymal signaling in a given patient and skin location as in every normal hair follicle.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Ramin Massoumi[1], Maurizio Podda[2], Reinhard Fässler[1] and Ralf Paus[1,3]

[1]Department of Molecular Medicine, Max-Planck-Institute for Biochemistry, Martinsried, Germany; [2]Department of Dermatology, University of Frankfurt, Frankfurt, Germany and [3]Department of Dermatology, University Hospital Schleswig-Holstein, Campus Lübeck, University of Lübeck, Lübeck, Germany.
E-mail: massoumi@biochem.mpg.de

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