Loss of heterozygosity at cylindromatosis gene locus, CYLD, in sporadic skin adnexal tumours

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Abstract

**Aim**—The gene for familial cylindromatosis (CYLD) has been localised to chromosome 16q, and has recently been cloned. Loss of heterozygosity (LOH) at 16q has also been demonstrated in sporadic cylindromas. The aim of this study was to investigate whether CYLD plays a role in the development of other skin appendage tumours.

**Methods**—A total of 55 cases of skin adnexal tumours, comprising 12 different types, and a control group of 14 squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs) were studied. Three microsatellites (D16S407 (16p), D16S304 (16q), and D16S308 (16q)) were analysed for LOH after microdissection from paraffin wax embedded sections using laser capture microdissection.

**Results**—In keeping with previous data, a proportion of cylindromas exhibited LOH at markers on 16q, but not at 16p. The skin adnexal tumours showing a similar pattern included apocrine hydrocystomas, eccrine spiradenomas, and sebaceous adenomas. One case of syringoma showed LOH at 16q, and a further case at 16p, but not 16q. One case of eccrine hydrocystoma showed loss at 16p, but not 16q. The remaining tumours were either negative or non-informative. All tumours in the control group were either negative or non-informative. Except for a single case of BCC showing LOH at 16q.

**Conclusion**—CYLD may be involved in the development of skin adnexal tumours other than cylindromas.

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Keywords: cylindromatosis locus CYLD; loss of heterozygosity; skin adnexal tumours

The two most common skin tumours are squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs), which together account for 95% of non-melanoma skin cancers.1 Although both are keratinocyte derived tumours, they have important biological and clinical differences. BCCs are typically slow growing, locally aggressive tumours that very rarely metastasise. In contrast, SCCs generally grow faster, are also locally invasive, but have metastatic potential. Genetic alterations on chromosomes 3, 9, 13, and 17 have been described in SCCs, whereas BCCs show alterations predominantly on chromosome 16q.2

Adnexal skin tumours are an uncommon but related group of benign neoplasms, which differentiate towards epidermal appendages rather than surface epidermis. They are a perplexing and difficult group of tumours, comprising different morphological types with confusing nomenclature and overlapping histological appearances. The overlap in histological features has led some authors to postulate that adnexal skin tumours represent aberrant differentiation from pluripotent basaloid cells.3 Features of these neoplasms include a tendency to develop multiple tumours, especially within a familial setting, low incidence of malignant transformation, and a good prognosis with little morbidity.

The gene for familial cylindromatosis (CYLD) has been localised to chromosome 16q using linkage analysis,4 and loss of the wild-type allele in tumours is consistent with the gene acting as a tumour suppressor. CYLD has recently been cloned5 using standard fine mapping and a positional cloning technique. Germline mutations have been detected in families affected by cylindromatosis, and somatic mutations have been seen in both sporadic and familial cylindromas. Loss of heterozygosity (LOH) at this site has also been demonstrated in a high proportion of sporadic cylindromas and it seems to be the only important site of genetic alteration.6 Studies on skin adnexal tumours other than cylindromas are sparse, and few genetic alterations have been demonstrated in these lesions.7 We hypothesised that the gene for cylindromatosis might play a role in the development of all skin appendage tumours and have therefore investigated LOH on chromosome 16 in a range of such tumours.

**Materials and methods**

A total of 55 cases of skin adnexal tumours were obtained from the histopathology archives of the Royal Victoria Infirmary, Newcastle Upon Tyne Hospitals NHS Trust. All cases were identified and reviewed by an experienced histopathologist (NL) with an interest in dermatopathology. Formalin fixed, paraffin wax embedded tissues comprising cylindroma (four), trichoepithelioma (two), apocrine hydrocystoma (four), syringoma (seven), eccrine spiradenoma (seven), sebaceous adenoma (four), eccrine hydrocystoma (five), trichoepithelioma (eight), eccrine poroma (eight), trichilemmoma (four), eccrine carcinoma (one), and desmoplastic trichoepithelioma (one) were obtained. Five SCCs and nine BCCs were also studied because 16q LOH is not a feature of these tumours.

Tumour cells and normal tissue (overlying normal epidermis, lymphoid aggregates, and
inflammatory infiltrates) were obtained using laser capture microdissection1 (PixCell II; Arcturus, Mountain View, California, USA) from 5 µm sections stained with haematoxylin and eosin. Tissue was pooled from up to five serial sections depending on the size of the lesion. DNA was extracted by standard protocols in a total volume of 15–20 µl using proteinase K, as described previously.2

Three microsatellite markers—D16S407 (16p) (forward 5’-CTCGCCTGGGTTACGTTAAT-3’, reverse 5’-AGATGAGGATGCTTTC-3’), D16S304 (16q) (forward 5’-GTCAAGCTGATTAAGGAAGTTAAGAAAAG-3’, reverse 5’-GATCAGATGAGATAAGGCGATTTTTG-3’), and D16S308 (16q) (forward 5’-CAGCCAGGATGTAAGGCAGCAGCT-3’, reverse 5’-TGGTGGCAGAGTGAGACCTGCTT-3’)—were used in our study. The 16q markers were chosen because these lie close to the region of the recently cloned CYLD gene.3 The 16p marker was used as a negative control. The forward strand of each primer pair was fluorescently labelled using either the FAM or TET dye (Oswel, Southampton, UK and Genosys, Cambridge, UK, respectively). The polymerase chain reaction (PCR) was performed in a total reaction volume of 12.5 µl. The PCR mixture contained 1× PCR buffer (Gibco BRL Paisley, UK); 200µM each dNTP; 1.25 pmol (D16S407), 0.75 pmol (D16S304), or 0.63 pmol (D16S308) of each primer; 1.5mM MgCl₂ (D16S407 and D16S304) or 1.0mM MgCl₂ (D16S308); 0.25 U of platinum Taq DNA polymerase (Gibco BRL); and 2 µl (from total extraction volume of 15–20 µl) of DNA template. Amplification was carried out for 40 cycles at 94°C for one minute, 55°C for one minute, and 72°C for one minute, with a final extension step at 72°C for 10 minutes.

The products were analysed for LOH on an ABI 373A automated fluorescent DNA sequencer (Applied Biosystems Warrington, UK) using Genescan analysis software (version 2.1) (Applied Biosystems) and imported into Genotyper (version 1.1) (Applied Biosystems). The data were assessed by comparing tumour to normal allele intensity ratios, and a value of ≤ 0.5 was assigned as indicative of LOH.4

Table 1 Loss of heterozygosity (LOH) in skin adnexal tumours

<table>
<thead>
<tr>
<th>Type (no.)</th>
<th>16p (D16s407)</th>
<th>16q (D16s304)</th>
<th>16q (D16s304)</th>
<th>Overall LOH at 16q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylindroma (4)</td>
<td>0/4 (0%)</td>
<td>2/4 (50%)*</td>
<td>2/3 (67%)*</td>
<td>75%</td>
</tr>
<tr>
<td>Trichoepithelioma (2)</td>
<td>0/1 (0%)</td>
<td>NI</td>
<td>1/2 (50%)</td>
<td>50%</td>
</tr>
<tr>
<td>Apocrine hydrocytoma (4)</td>
<td>0/3 (0%)</td>
<td>0/3 (0%)</td>
<td>1/3 (33%)</td>
<td>33%</td>
</tr>
<tr>
<td>Eccrine spiradenoma (7)</td>
<td>0/5 (0%)</td>
<td>0/5 (0%)</td>
<td>1/5 (20%)</td>
<td>20%</td>
</tr>
<tr>
<td>Sebaceous adenoma (4)</td>
<td>0/2 (0%)</td>
<td>1/3 (33%)</td>
<td>1/3 (33%)</td>
<td>66%</td>
</tr>
<tr>
<td>Syringoma (7)</td>
<td>1/5 (20%)</td>
<td>0/3 (0%)</td>
<td>1/5 (20%)</td>
<td>20%</td>
</tr>
<tr>
<td>Eccrine hydrocytoma (5)</td>
<td>1/4 (25%)</td>
<td>0/5 (0%)</td>
<td>0/4 (0%)</td>
<td>0%</td>
</tr>
<tr>
<td>Trichofolliculoma (8)</td>
<td>0/6 (0%)</td>
<td>0/8 (0%)</td>
<td>0/5 (0%)</td>
<td>0%</td>
</tr>
<tr>
<td>Eccrine poroma (8)</td>
<td>0/6 (0%)</td>
<td>0/7 (0%)</td>
<td>0/7 (0%)</td>
<td>0%</td>
</tr>
<tr>
<td>Trichilemmoma (4)</td>
<td>0/5 (0%)</td>
<td>0/3 (0%)</td>
<td>0/1 (0%)</td>
<td>0%</td>
</tr>
<tr>
<td>Eccrine carcinoma (1)</td>
<td>NI</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
<td>0%</td>
</tr>
<tr>
<td>Desmoplastic trichoepithelioma (1)</td>
<td>1/0 (0%)</td>
<td>NI</td>
<td>1/0 (0%)</td>
<td>0%</td>
</tr>
<tr>
<td>Basal cell carcinoma (9)</td>
<td>0/5 (0%)</td>
<td>1/8 (13%)</td>
<td>0/5 (0%)</td>
<td>13%</td>
</tr>
<tr>
<td>Squamous cell carcinoma (5)</td>
<td>0/3 (0%)</td>
<td>0/4 (0%)</td>
<td>0/3 (0%)</td>
<td>0%</td>
</tr>
</tbody>
</table>

The data are presented as number of cases exhibiting LOH/number informative for that marker (%).

1One case showed LOH at both 16q markers.
2NI, not informative; no, number of cases studied.

Results

Fifty five skin adnexal and 14 keratinocyte derived tumours (BCCs, SCCs) were analysed for LOH on chromosome 16 using three microsatellite markers. Table 1 summarises the data. In keeping with previous data, three of the four cylindromas exhibited LOH at one or other of the 16q markers (D16S308 or D16S304), but not at 16p (D16s407) (fig 1). The skin adnexal tumours showing a similar pattern (LOH at 16q but not at 16p) included one of three trichoepitheliommas, one of three apocrine hydrocytomomas, one of five eccrine spiradenomas, and two of three sebaceous adenomas (fig 2). One of five syringomas exhibited LOH at 16q, and one further case showed LOH at 16p, but not 16q. One case of eccrine hydrocytoma also showed loss at 16p, but not 16q. All cases of trichofolliculoma, eccrine poroma, trichilemmoma, eccrine carcinoma, and desmoplastic trichoepithelioma were either non-informative or negative at the markers studied. One of eight informative cases of BCC showed LOH at 16q (D16S308), but not at the second 16q marker or at 16p. All five SCCs were either negative or non-informative for all markers.

Discussion

Adnexal skin tumours are an uncommon but related group of neoplasms, which differentiate towards epidermal appendages rather than surface epidermis. They can differentiate towards several different cell lineages, namely hair follicles, sebaceous glands, apocrine glands, and eccrine glands. Individual tumours...
LOH at CYLD in sporadic skin adnexal tumours

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There is a loss of the 151 bp allele (allele ratio of 0).

Figure 2 (A) Histology of sebaceous adenoma (haematoxylin and eosin stain; original magnification, ×200). (B) Loss of heterozygosity at 16q (D16S304).
There is a loss of the 151 bp allele (allele ratio of 0).
can also exhibit more than one type of
differentiation, further complicating the no-
menclature. Other features in common include
a tendency for the development of multiple
tumours and the occurrence of familial syn-
dromes.4,5

Hereditary cylindromatosis is an autosomal
dominant disease characterised by the develop-
ment of multiple skin cylindromas, a type of
adnexal tumour. Occasionally, other skin adn-
exal tumours, such as trichoepithelioma and
spiradenoma have been reported.10 11 The gene
responsible was localised in 1995 to chromo-
some 16q12–q13.4 Consistent loss of the wild-
type allele was observed, indicating that the
gene is probably a tumour suppressor. Unlike
familial cylindromatosis, sporadic cylindromas
occur later in life and are less likely to be mul-
tiple. LOH at 16q has also been demonstrated
in sporadic cylindromas,9 and it appears that it
might be the only tumour suppressor gene
involved in the syndrome. Recently, the familial
cylindromatosis gene, CYLD, has been identi-
fied and cloned by detecting 21 different
germline mutations in families affected by
cylindromatosis, and six somatic mutations
from both familial and sporadic cylindromas.5
The gene for multiple familial trichoepithe-
lioma has been mapped to chromosome
9p21.12 Clearly, it would be interesting to
investigate whether trichoepitheliomas arising
within the setting of familial cylindromatosis
show alterations at this locus.

In our study, we have shown that LOH at
16q is present in several morphologically
distinct skin adnexal tumours, including cylin-
dromas, sebaceous adenomas, trichoepitheli-
omas, apocrine hydrocystomas, syringomas, and
eccrine spiradenomas. The finding of LOH in
these lesions showing diverse adnexal differen-
tiation provides tentative evidence for their ori-
gin from a common pluripotential cell. Fur-
thermore, the data provide support for the
hypothesis that CYLD plays a role in the
development of at least a proportion of skin
adnexal tumours. Further support for this
hypothesis comes from a report of 16q22 loss
in an eccrine spiradenoma,13 although this was
an unusual case with lymph node metastases
(but not distant metastases).

Takata and co-workers2 have examined a
range of adnexal tumours for LOH at multiple
loci, but did not investigate chromosome 16q.
The overall frequency of LOH in their series
was 1.6% (four of 247 informative loci).
Examples include LOH at 17q in sebaceous
epithelioma and eccrine porocarcinoma, and
LOH at 9q in trichoepithelioma. These altera-
tions in skin adnexal tumours were different to
those identified in BCCs and SCCs.

The only other important data in adnexal
tumours has been in p53 expression. p53 accu-
mulation was found in 10 of 14 sweat gland
carcinomas and three of 60 sweat gland adeno-
as.14 15 p53 expression has been documented in
spiradenomas, but this is only in the malignant
portions of malignant spiradenomas.15 The
benign components of the lesion remain nega-
tive. Malignant sweat gland tumours have a
door prognosis and the finding of abnormal
p53 protein expression might reflect increasing
genetic instability with accumulation of chro-
mosome damage.

In Muir Torre and Cowden’s syndromes,
multiple skin adnexal tumours are associated
with underlying malignancy—colon cancer in
Muir Torre syndrome, breast and thyroid
carcinomas in Cowden’s syndrome.16 17 Cylin-
dromatosis does not have such systemic mani-
festations, and defects in mismatch repair
enzymes (Muir Torre syndrome) or mutations
in PTEN (Cowden’s syndrome) are not likely
to play an important role in this disease.

Pilomatricomas are skin tumours of un-
known origin. A recent paper18 suggests that
they arise from hair matrix cells and that many
have mutations in the β-catenin gene. The role
of β-catenin in skin adnexal tumours is
unknown.

SCCs are easy to distinguish from adnexal
tumours at a morphological and molecular
level. Compared with BCCs and skin adnexal
tumours, they exhibit LOH at multiple sites
including 3p, 9p, 9q, 13, and 17p.19 A relation
between the accumulation of genetic abnor-
malities and the clinical behaviour of a
neoplasm has been suggested.20 This might
account for the relatively more aggressive
behaviour and metastatic potential of SCCs.
In our series, a single case of BCC showed
LOH at one marker on 16q. This case has been
re-reviewed in light of these data. It is still felt
to be a BCC, although there are some unusual
features in the form of organoid growth pattern
and focal adnexal differentiation. There is no
evidence in the literature to suggest that 16q
alterations play a role in the development of
BCCs. Molecular genetic analysis has demonstrated mutation at 9q22–31 in 70% of sporadic BCCs. This mutation is not found in adnexal tumours. Whether the 16q LOH is a pathogenetic alteration or a non-specific random deletion is unclear in this case. Most of the data in the literature suggest that the evolution of BCCs and skin adnexal tumours occurs via different genetic pathways.

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