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 adenoma. 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.

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), trichofolliculoma (eight), eccrine poroma (eight), trichillemmoma (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 dermis) from each case were microdissected by a single histopathologist (NL) with an interest in dermatopathology. The DNA was extracted from each sample using standard methods. LOH was assessed using three polymorphic microsatellite markers on chromosome 16: D16S407 (16p), D16S304 (16q), and D16S308 (16q) were analysed for LOH after microdissection from paraffin wax embedded sections using laser capture microdissection.
inflammatory infiltrates) were obtained using laser capture microdissection (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.

Three microsatellite markers—D16S407 (16p) (forward 5'-CTCGCCGCTGGTGACACAGTAT3'; reverse 5'-GGTCCGCTGGTGACACAGTAT3'), D16S304 (16q) (forward 5'-CTCGCACTGGAGGTAAGAAAAAGC3'; reverse 5'-GGTCCGCTGGTGACACAGTAT3'), and D16S308 (16q) (forward 5'-CTCGCGCTGGTGACACAGTAT3'; reverse 5'-GGTCCGCTGGTGACACAGTAT3')—were used in our study. The 16q markers were chosen because these lie close to the region of the recently cloned CYLD gene. 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 (Oswell, 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); 200 µM each dNTP; 1.25 pmol (D16S407), 0.75 pmol (D16S304), or 0.63 pmol (D16S308) of each primer; 1.5 mM MgCl2 (D16S407 and D16S304) or 1.0 mM 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.

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 (D16s308)</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 hydrocystoma (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 hydrocystoma (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>0/3 (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>0/1 (0%)</td>
<td>NI</td>
<td>0/1 (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 (%).

*One case showed LOH at both 16q markers.

NI, not informative; no, number of cases studied.

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

... responsible was localised in 1995 to chromosome 9p21.12 Clearly, it would be interesting to investigate whether trichoepitheliomas arising from both familial and sporadic cylindromas... 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 eccrine spiradenomas. The finding of LOH in these lesions showing diverse adnexal differentiation provides tentative evidence for their origin from a common pluripotential cell. Furthermore, 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, although this was an unusual case with lymph node metastases (but not distant metastases).

Takata and co-workers 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 alterations 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 accumulation was found in 10 of 14 sweat gland carcinomas and three of 60 sweat gland adenomas. p53 expression has been documented in spiradenomas, but this is only in the malignant portions of malignant spiradenomas. The benign components of the lesion remain negative. Malignant sweat gland tumours have a poor prognosis and the finding of abnormal p53 protein expression might reflect increasing genetic instability with accumulation of chromosome 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. Cylin-dromatosis does not have such systemic manifestations, 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 unknown origin. A recent paper 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. A relation between the accumulation of genetic abnormalities and the clinical behaviour of a neoplasm has been suggested. 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 a mutation at 9q22–31 in 70% of sporadic BCCs. This mutation is not found in adenoid 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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