Advances in Brief

Increased Frequency of Spontaneous Skin Tumors in Transgenic Mice Which Overexpress Ornithine Decarboxylase

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Abstract

Ornithine decarboxylase, a critical regulatory enzyme for polyamine biosynthesis, is highly inducible by growth-promoting stimuli in mouse epidermis but the enzyme level is only transiently elevated due to rapid turnover of the protein. Here we report that constitutive overexpression of the enzyme in the skin of transgenic mice causes several phenotypic abnormalities. Effects observed include development of dermal follicular cysts, excessive skin wrinkling, enhanced nail growth, alopecia, and spontaneous tumor development. These results indicate that up-regulation of polyamine biosynthesis can profoundly disturb skin homeostasis and alter susceptibility to neoplastic development.

Introduction

ODC catalyzes the decarboxylation of L-ornithine to the diamine putrescine. Putrescine and the polyamines spermidine and spermine are cellular polycations essential for normal cell proliferation and differentiation. ODC expression in mammalian tissues is usually tightly regulated, although neoplastic cells often express high levels of the enzyme (1). Many factors other than the level of ODC activity, including other polyamine biosynthetic and degradative enzymes, the intracellular ornithine concentration, and polyamine excretion, exist to regulate intracellular polyamine concentrations. Nevertheless, one approach to study the possible deleterious effects of sustained high levels of polyamines on cell proliferation, differentiation, and neoplastic transformation is to up-regulate ODC expression constitutively. Several groups have used transfection and retroviral infection techniques to overexpress an exogenous ODC cDNA in various cell types (2-5). In addition, Halmekyto et al. (6) have developed transgenic mice that overexpress the human ODC gene in certain tissues such as brain and testis. Only a modest increase in the basal level of ODC was reported for epidermis in these mice and no phenotypic effects in the skin of untreated mice were observed although the mice were more susceptible to a skin chemical carcinogenesis protocol (7). In the present study, we decided to target ODC overexpression to epidermis. This tissue was chosen because of its high ornithine content, which should facilitate polyamine accumulation if ODC is overexpressed, and its utility as a well-characterized model of multistage carcinogenesis (9-11). Moreover, we decided to target ODC expression to a specific subpopulation of epidermal keratinocytes via the use of a bovine keratin IV (homologous to mouse and human keratin 6; hereafter referred to as K6) promoter-driven ODC transgene.

Materials and Methods

Production and Characterization of Transgenic Mice. Transgenic mice were generated by DNA microinjection of fertilized B6C3F2 oocytes by using standard techniques (13). The transgene was a 20-kb fragment derived from Ncol digestion of a pBR322-based vector containing a K6 minimal promoter upstream of a murine ODC cDNA [from -69 to +1682 bp of the ODC cDNA sequence (14), containing an introduced stop codon at position 427]. Genomic DNA was isolated from the tails of potential founder mice and subjected to Southern blot and PCR analyses to identify mice bearing the transgene. For Southern analyses, DNAs digested with EcoRI were electrophoresed on 1% agarose gels, blotted onto nitrocellulose filters, and sequentially probed by using a 1.75-kb ODC cDNA fragment and a DNA probe derived from the K6 promoter. Both probes were radiolabeled with 32P by random prime labeling and hybridized to the same 2.8-kb EcoRI fragment containing transgene sequence. For PCR analyses, the S1-primer, P1, was a K6 promoter sequence (5'-GCACAGAGGGGACAAATTACA-3') and the 3-primer, P2, was an oligonucleotide the sequence of which corresponded to an ODC coding sequence (5'-TGCCATGTCAAACACACAGCGG-3'). The amplified 1.2-kb fragment, therefore, spanned the junction between the K6 promoter and the ODC sequence and was only detected in mice bearing the transgene. Control PCR reactions were performed by using two other primers designed to amplify exon 11 of the endogenous ODC gene (5'-GGGATCTTAAAGAGAA-CAATG-3' and 5'-CCACCACCAAGCAGGAAAATCA-3').

Histology and Immunocytochemistry. For histological analysis, tissues were fixed overnight in 10% neutral buffered formalin or Fekete's solution (60% EtOH, 3.2% formaldehyde, and 0.75 M acetic acid), embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin. For immunolocalization of ODC, skin sections were incubated with an anti-ODC antiserum and specific staining was detected with an ABC Vectastain kit (Vector Laboratories, Burlingame, CA) as described previously (15).

Biochemical Methods. To prepare tissue extracts for ODC activity and polyamine determinations, transgenic mice were killed, and the skin was excised and exposed to 55°C dH2O for 20 s. The epidermis was subsequently scraped off, and portions were placed either in buffer A [25 mM Tris-HCl (pH 7.5), 2.5 mM DTT, and 0.1 mM EDTA] or 0.2 N perchloric acid. The remaining dermis was minced thoroughly with scissors and portions were placed in buffer A or 0.2 N perchloric acid. Tissues were homogenized in a Polytron homogenizer and centrifuged at 20,000 × g for 20 min. The resulting supernatants were assayed for ODC activity (buffer A extract) or polyamine levels (0.2 N perchloric acid extract) as described (7). Normal littermates were handled similarly, except preshaved animals were depilated for 5 min immediately after killing. For ODC, results are expressed as units/mg protein, where 1 unit = 1 nmol CO2 liberated/h. For polyamines, results are expressed as nmol polyamine/mg DNA.

Results and Discussion

A DNA fragment in which a mutant mouse ODC cDNA (containing a premature stop codon at position 427 resulting in a carboxyl-
Fig. 1. Production of transgenic mice which overexpress ODC. A, a pBR322-based vector containing a keratin 6 minimal promoter upstream of a murine ODC cDNA [from −69 to +1682 bp of the ODC cDNA sequence (13), containing an introduced stop codon at position 427] was prepared. For DNA microinjection, the plasmid was digested with NcoI, and the linearized 20-kb fragment was purified on a 1% agarose gel. B, genomic DNAs from four founder mice and three non-transgenic littermates were digested with EcoRI and subjected to Southern analysis. The probe used was a 1.75-kb ODC cDNA fragment. Lane 9 represents the plasmid DNA used for microinjection. A 2.8-kb band of varying intensity is present only in DNA derived from founder mice. C, the same blot shown in B was stripped and rehybridized with a probe derived from the K6 promoter. D, PCR analysis of tail DNA from founder mice and normal-appearing littermates. Primers derived from the K6 promoter (P1) and the ODC cDNA (P2) were used to amplify a 1.2-kb fragment only in founder mice (Lanes 1-6). Lanes 1-3, normal littermates, whereas Lanes 4-6 are reactions from founder animals. The same DNAs were used in a separate PCR reaction by using primers designed to amplify exon 11 of the endogenous ODC gene (Lanes 7-12). kbp, kilobase pair.

Fig. 2. Skin of normal sibling (A) and transgenic mouse (B) showing the replacement of the s.c. fat tissue by the large follicular cysts that extend to the muscle layer. C, a higher power view of transgenic skin. Note that the cysts are variable in size and are lined by well-differentiated keratinocytes. D, immunohistochemistry (performed as described in Ref. 15 at an antibody dilution of 1:3000) shows expression (brown color) of ODC in the dermis confined to the cell layer lining the cysts (D), with no positive staining in the epidermis. A and B, × 125; C and D, × 250.
complete hair loss, including vibrissae, by 2 months of age. With
was complete in six of eight founders, but the transgenic progeny of
beginning at 2-3 weeks of age, and excessive nail growth. Hair loss
weight, a normal first hair cycle, followed by progressive hair loss
founders analyzed. Founder mice were characterized by smaller birth
was microinjected into the male pronucleus of fertilized B6C3F2
terminal truncation) placed downstream of the K6 promoter (Fig. 1A)
was microinjected into the male pronucleus of fertilized B6C3F2
otic expression was actually due to transgene expression, Western
ysis revealed that multiple copies of
37°C. The L-ornithine concentration used was 0.125 mm.
37°C. The L-ornithine concentration used was 0.125 mm.
Table 1 Ornithine decarboxylase levels in transgenic mouse skin
A unit of ODC activity is equivalent to 1 nmol CO2 liberated from L-ornithine/h at
Table 2 Polyamine levels in normal versus transgenic skin
Polyamine levels were determined as described in Ref. 5.
many terminal truncation) placed downstream of the K6 promoter (Fig. 1A)
can be expected to have a much longer in vivo half-life than a full-length ODC (16). Eight founder mice were identified by
polyamine levels, especially putrescine and spermidine, were greatly elevated in transgenic mouse dermis,
ly expressed to yield a major 4207 molecular weight protein (Fig. 3). The only protein detected in extracts
nerve fibers in the dermis. Polyamine levels, especially putrescine and spermidine, were greatly elevated in transgenic mouse dermis,
Table 2 Polyamine levels in normal versus transgenic skin
Polyamine nmol/mg DNA in

<table>
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<tr>
<th>Mouse</th>
<th>Epidermis</th>
<th>Dermis</th>
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<tbody>
<tr>
<td>Founder 1</td>
<td>76.9</td>
<td>349</td>
</tr>
<tr>
<td>Littermates (n = 2)</td>
<td>66.2</td>
<td>228</td>
</tr>
<tr>
<td>Founder 2A</td>
<td>66.2</td>
<td>228</td>
</tr>
<tr>
<td>Littermates (n = 2)</td>
<td>66.2</td>
<td>228</td>
</tr>
<tr>
<td>Founder 3</td>
<td>179</td>
<td>645</td>
</tr>
<tr>
<td>Littermate</td>
<td>44.7</td>
<td>478</td>
</tr>
<tr>
<td>Founder 4</td>
<td>176</td>
<td>356</td>
</tr>
<tr>
<td>Littermate</td>
<td>103</td>
<td>760</td>
</tr>
<tr>
<td>Founder 5</td>
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</tr>
<tr>
<td>Littermate</td>
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<td>443</td>
</tr>
<tr>
<td>Founder 6B</td>
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<td>346</td>
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<tr>
<td>Littermate</td>
<td>80.6</td>
<td>346</td>
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</table>

* Pu. putrescine; Spd, spermidine; Sp, spermine.
# ND, not determined.
ODC OVEREXPRESSION IN MICE AND SKIN TUMOR FREQUENCY

results are summarized in Table 3. The absolute values of ODC-specific activity were extremely high in all tumors. It is worth noting that the mean value shown in Table 3 is 11-fold higher than the mean of a series of epidermal papillomas induced by a 7,12-dimethylbenz(a)anthracene/12-O-tetradecanoylphorbol-13-acetate-initiation promotion protocol recently analyzed by us (8). When the tumor illustrated in Fig. 4E was analyzed for ODC expression immunocytochemically, intense staining was observed throughout the tumor, particularly in the suprabasal cells. Western analysis indicated that ODC overexpression in most cases was due to elevated transgene expression, although significant coexpression of the endogenous ODC gene was detected in some tumors (data not shown). Most of the tumors arose in older mice and did not regress. Because of the relatively long interval between onset of transgene expression and tumor appearance, as well as the low multiplicity of tumors/mouse, it is likely that a second genetic or epigenetic event...
has occurred that cooperates with ODC overexpression to facilitate tumor development. Consistent with this idea is the recent report (5) that constitutive overexpression of ODC is not sufficient to induce tumors in normal epithelial or fibroblast cells but is sufficient to enhance tumor development in initiated keratinocyte cell lines. There are several obvious candidates for a “second hit,” such as mutations in proto-oncogenes or loss of functional tumor suppressor genes. It will be of interest to determine the nature and diversity of genetic alterations that can cooperate with ODC overexpression in this model.

Our results indicate that ODC overexpression targeted to the outer root sheath cells of the hair follicle can profoundly alter skin structure and function. One of the earliest phenotypes observed is the disruption of the hair cycle. At present, we have little information regarding the time of onset of ODC overexpression, although small follicular cysts are present in transgenic skin as early as 2 weeks of age (data not shown). Because the first hair cycle is normal, it is likely that ODC transgene expression does not become significant until sometime after birth. Although there are numerous mutations in the mouse that cause hair loss (19), the functions of most of the genes involved are not known. The mice described here could be a useful model for characterizing genes regulated by increased polyamine levels, for studies on the diversity of genetic alterations that can cooperate with ODC overexpression in this model.

Acknowledgments

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References


Table 3 ODC activity in spontaneous skin tumors arising in transgenic mice

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Mouse</th>
<th>ODC-specific activity</th>
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<tbody>
<tr>
<td>A1</td>
<td>Founder</td>
<td>36.4</td>
</tr>
<tr>
<td>B1</td>
<td>Fl1</td>
<td>120</td>
</tr>
<tr>
<td>C1</td>
<td>Fl1</td>
<td>103</td>
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<tr>
<td>D1</td>
<td>Fl1</td>
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<tr>
<td>F1</td>
<td>Fl1</td>
<td>146</td>
</tr>
<tr>
<td>G1</td>
<td>Founder</td>
<td>34.0</td>
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</table>

Mean ± SEM 83.6 ± 18.0
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