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Attenuation of UVR-induced vitamin D_3 synthesis in a mouse model deleted for keratinocyte lathosterol 5-desaturase

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ABSTRACT

The lower risk of some internal cancers at lower latitudes has been linked to greater sun exposure and consequent higher levels of ultraviolet radiation (UVR)-produced vitamin D_3 (D_3). To separate the experimental effects of sunlight and of all forms of D_3, a mouse in which UVR does not produce D_3 would be useful.

To this end we have generated mice carrying a modified allele of *sterol C5-desaturase* (*Sc5d*), the gene encoding the enzyme that converts lathosterol to 7-dehydrocholesterol (7-DHC), such that *Sc5d* expression can be inactivated using the Cre/lox site-specific recombination system. By crossing to mice with tissue-specific expression of Cre or $CreER^2$ (Cre/estrogen receptor), we generated two lines of transgenic mice. One line has constitutive keratinocyte-specific inactivation of *Sc5d* ($Sc5d^{k14KO}$). The other line ($Sc5d^{k14KOi}$) has tamoxifen-inducible keratinocyte-specific inactivation of *Sc5d*.

Mice deleted for keratinocyte *Sc5d* lose the ability to increase circulating D_3 following UVR exposure of the skin. Thus, unlike in control mice, acute UVR exposure did not affect circulating D_3 level in inducible $Sc5d^{k14KOi}$ mice.

Keratinocyte-specific inactivation of *Sc5d* was proven by sterol measurement in hair – in control animals lathosterol and cholesta-7,24-dien-3β-ol, the target molecules of SC5D in the sterol biosynthetic pathways, together constituted a mean of 10% of total sterols; in the conditional knockout mice these sterols constituted a mean of 56% of total sterols. The constitutive knockout mice had an even greater increase, with lathosterol and cholesta-7,24-dien-3β-ol accounting for 80% of total sterols.

In conclusion, the dominant presence of the 7-DHC precursors in hair of conditional animals and the lack of increased circulating D_3 following exposure to UVR reflect attenuated production of the D_3 photochemical precursor 7-DHC and, consequently, of D_3 itself. These animals provide a useful new tool for investigating the role of D_3 in UVR-induced physiological effects and, more broadly, for investigations of the cholesterol synthetic pathway in the skin and other targeted tissues.

1. Introduction

In 1980 Cedric and Frank Garland first suggested that the latitudinal gradient of decreased cancer deaths at lower latitudes, known in particular for cancers of the colon and breast, might be due to the anti-cancer effects of sunlight-produced vitamin D_3 (D_3) [1]. Sparked in large part by their hypothesis, investigation of D_3 and cancer has been an area of intense study with more than 9000 papers on this subject listed in PubMed. We describe here our success in constructing a transgenic mouse to facilitate study of the effects of ultraviolet radiation (UVR) independent of D_3 production and its hydroxylated derivatives. Specifically, we have engineered a conditional mouse allele designed to allow tissue-specific deletion of the gene encoding sterol C5-desaturase (*Sc5d*), the enzyme responsible for the production of 7-dehydrocholesterol (7-DHC).

Deficiency of SC5D (lathosterolosis) (OMIM 607330) joins DHCR7 deficiency (Smith-Lemli-Opitz syndrome) and 24-dehydrocholesterol reductase deficiency (desmosterolosis) as a disorder of post-squalene deficient cholesterol synthesis. Patients with lathosterolosis have elevated serum concentrations of lathosterol; four individuals with this condition have been reported, of whom only two survived infancy [2,3]. Surviving individuals in all three conditions have multiple congenital defects and developmental delays.

Similarly, global homozygous deletion of the mouse *Sc5d* gene

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produces stillborn pups with intrauterine growth retardation, craniofacial abnormalities (including cleft palate and micrognathia), and limb patterning defects [3]. Many of the malformations (in humans and mouse models) are consistent with disruption during early vertebrate development of hedgehog signaling, which is influenced by cholesterol and its metabolites. Several different mechanisms for this influence have been described, among which the permissive and activating functions of direct binding of cholesterol to smoothened currently appear to be crucial [4–7]. Since global SC5D deficiency is lethal in mice, we have instead focused on constructing a model in which we use Cre/lox technology to achieve tissue- or stage-specific conditional inactivation, specifically deleting the *Sc5d* gene in keratinocytes. Herein, we report this construction and our characterization of mice lacking the *Sc5d* gene in keratinocytes, confirming the success of this approach by hair sterol analysis and by loss of ability to increase circulating D_3 following acute UVR exposure.

2. Materials and methods

2.1. Generation of Sc5d embryonic stem (ES) cells and $Sc5d^{tm1a(EUCOMM)Hmgu}$ mice

We utilized the European Conditional Mouse Mutagenesis (EUCOMM) [8] library of agouti C57Bl/6N (JM8A3.N1)[9] murine ES cells containing the PG00187_Z_8_B03 targeting vector integrated into the L1L2 Bact P cassette inserted in chromosome 9 and targeting exon 4 of the Sc5D gene, causing a reading frame shift and thereby likely triggering nonsense mediated decay of the aberrant transcript (Fig. 2A). For details see www.knockout.org [10]. Mice were generated at the University of California Davis [11] by blastocyst injection into C57Bl/6N mice of three ES cell clones, one of which produced germline transmitting $Sc5d^{tm1a(EUCOMM)Hmgu}$ founders (abbreviated as $Sc5d^{tm1a/+}$).

2.2. Treatment groups and procedures

2.2.1. Mouse care

The FLPo deleter mouse, 129S4/SvJae-$Gt(ROSA)26Sor^{tm2(FLP*)Sor}$/J, the keratinocyte-specific constitutive Cre recombinase-expressing mouse, Tg(KRT14-Cre)1Amc/J, and the FVB/NJ mice were from The Jackson Laboratory (JAX, Sacramento, stock nos. 007844, 004782, and 001800 respectively). Tamoxifen-inducible keratinocyte-specific Cre recombinase-expressing mice with a mutated ligand-binding domain for the human estrogen receptor (ER), K14-$CreER^2$ were originally from Pierre Chambon (University of Strasbourg) [12].

Mice were housed under standard conditions (fluorescent lighting 12 h per day, room temperature 23 °C–25 °C, and relative humidity 45–55%). Mice were maintained on standard diet (a normal D_3/normal minerals diet, D_3 1500 IU/kg, Ca 1%, Phosphate 0.7%; TD2018: Harlan, Madison, WI). We used 6 week-old mice for all studies unless otherwise specified in the text. For dietary studies mothers and enrollees were weaned onto and maintained on a D_3 depleted/normal minerals diet (D_3 0 U/kg, Ca 1%, Phosphate 0.7%; TD89123: Harlan, Madison, WI).

Animal care and use were in compliance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Children's Hospital Oakland Research Institute (CHORI).

2.2.2. Breeding and genotyping of mutant mice

To create specific Cre recombinase-mediated deletions within the

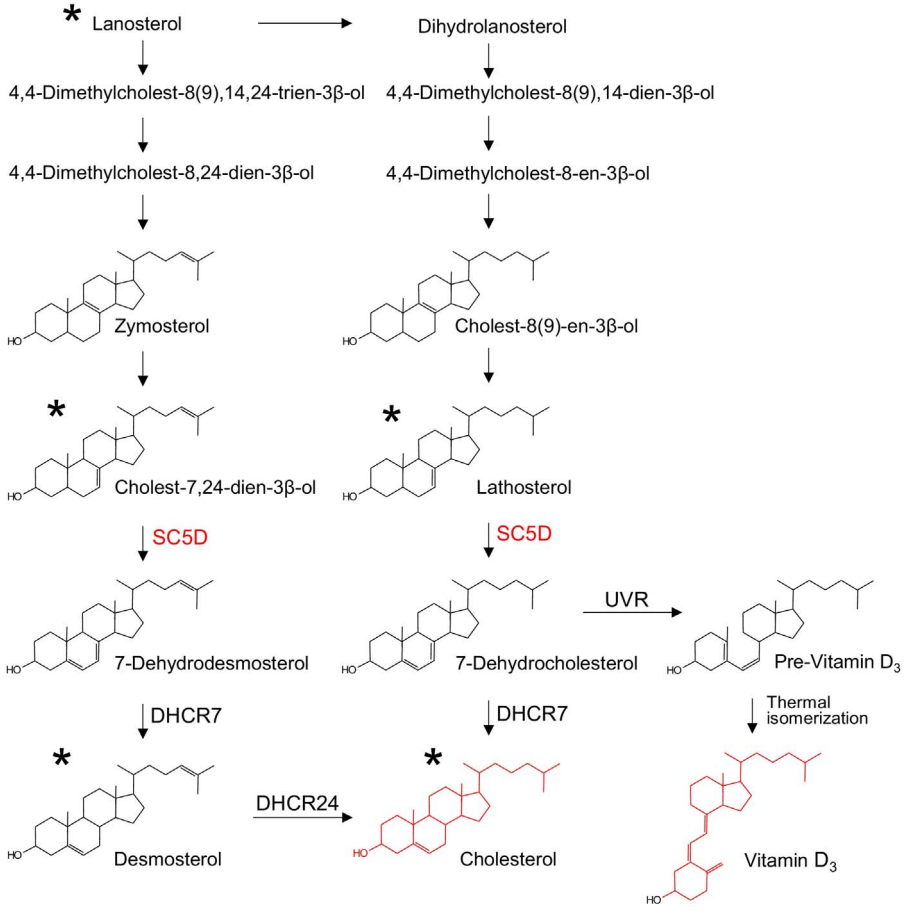

Fig. 1. Post squalene cholesterol and vitamin D_3 synthesis. Two synthetic pathways of cholesterol are shown, the Bloch pathway at left and the Kandutsch-Russell pathway at right. Vitamin D_3 synthesis in the skin is shown at right. The sterols marked with asterisks were identified and measured in this study.