Ectodysplasin signalling in cutaneous appendage development: Dose, duration and diversity

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The development of several skin appendages is guided by prenatal Ectodysplasin signalling. Cui et al. (this issue, 2009) report on the dose and duration of Ectodysplasin signalling required for the maintenance and morphogenesis of different appendages. They find that achievement of an intimate arrangement between epithelial and mesenchymal cell populations correlates with the acquisition of autonomy from Ectodysplasin stimulation.

Cutaneous appendage development

The skin arises from a simple sheet of embryonic ectoderm underlain by mesenchyme. The cells in this epithelial sheet are initially homogeneously distributed, but subsequently undergo clustering at specific locations to produce an array of placodes. Depending on their location on the body and time of formation these placodes develop into a number of diverse cutaneous appendages, including glands, teeth, and several types of hair follicle. Generation of a mature organ from the embryonic placode involves production of a downgrowth due to rapid epithelial proliferation followed by cellular differentiation to enable physiological functioning of the appendage (Schmidt-Ullrich and Paus, 2005).

The similar cellular rearrangements observed during development of multiple appendage types are underlain by the utilisation of common signalling pathways to guide cell behaviour. Though a regulatory efficiency is achieved by employing the same genes in multiple situations, an outcome of this shared genetic basis is that inherited conditions affecting appendage development tend to be syndromic, affecting multiple appendage types. One such condition, hypohidrotic ectodermal dysplasia

(HED), is characterised by a reduction in hair follicle development, the growth of a few, misshapen teeth and the absence of eccrine sweat glands.

HED is caused by mutation of genes encoding components of a TNF-like signalling pathway. Activation of this pathway is initiated by binding of the TNF-like ligand Ectodysplasin (EDA) to its transmembrane receptor EDAR. EDAR then connects to a canonical TNF signalling cascade through a dedicated adapter protein, ultimately leading to stimulation of NF-κB. Mutation of *EDA* is the most common cause of HED, which typically presents in boys due to its presence on the X-chromosome (Mikkola, 2008). Interestingly, not only is this signalling system common to a range of ectodermal appendages, but is required for appendage formation across the vertebrates, from human to fish (Harris *et al.*, 2008). With the breadth of model organs and organisms available for study, together with a well characterised signalling pathway, the basic questions regarding EDA action are moving on to examination of the quantity and timing of its action needed to guide normal development.

Critical periods and critical durations in appendage development

Cutaneous appendages form in a temporal sequence, with only one type of appendage produced in a particular skin region at a given time. For example, in the mouse three distinct waves of hair follicle formation produce the three different types of hair follicle in the adult. From embryonic day 14 (E14) to E16 primary hair follicles are formed; these make the long guard hairs of the coat and do not develop in animals lacking *Eda*. In contrast, the secondary and tertiary follicles initiate from approximately E16 and E19, respectively, and produce distinct hair types. These later waves of folliculogenesis do occur in *Eda* mutant mice, though abnormal hairs are produced.

The elucidation of the molecular basis of X-linked HED has enabled therapeutic efforts aimed at EDA protein replacement during development. The efficacy of such experimental therapies relies on identifying the critical periods during development at which different tissues are capable of responding to EDA. Addressing this issue in a mouse HED model, Gaide and Schneider administered a bolus of a recombinant EDA fusion protein at different times during development and determined the degree of

phenotypic rescue in adult animals. They found that the critical period at which EDA action is required to produce a particular appendage generally matches the time at which development of that structure is normally initiated. Thus midgestational EDA administration effected a much broader rescue of mutant phenotypes than did postnatal EDA treatment (Gaide and Schneider, 2003).

Cui et al (Cui et al., 2009) have also addressed the timing of Eda action in appendage development, but from a very different perspective. Using a mouse model in which the only source of Eda is a transgene that can be switched off by administration of doxycycline, they have examined the critical duration of signalling required stabilise the development of incipient hair follicles and sweat glands. They find that withdrawing Eda at E15, when the skin is populated with primary hair follicle placodes, leads to a lack of these follicles in the adult, demonstrating a requirement for a duration of Eda signal beyond the initial stages of placode formation. Abolition of *Eda* expression at E17 or later, however, produced a normal complement of guard hairs in the adult coat. This acquisition of Eda autonomy correlates with the establishment of a dermal papilla precursor in close association with the epithelial downgrowth (Figure 1). Thus progression of hair follicle development shifts from a reliance on the widely produced Eda signal to a more intimate reciprocal communication between these two closely apposed cell populations.

Eccrine sweat glands develop slightly later in than primary hair placodes and in mouse form only on the footpads. Despite these differences, an E19 sweat gland rudiment closely resembles an E17 hair follicle in the extent of epithelial downgrowth, but with the striking difference that there is no sign of an accompanying mesenchymal signalling centre. In contrast to the hair follicle rudiment, withdrawal of the Eda signal at this stage results in a failure of gland development, suggesting that the developing sweat gland's solitary epithelial cord requires sustained Ectodysplasin stimulation to reach maturity.

The ability to remove Eda also allows an examination of the cellular consequences of signal starvation. Eda withdrawal from the sweat gland rudiment leaves epithelial remnants lacking a definite organ identity stranded in the dermis. It would be interesting to determine the fate of cells in the primary hair placodes that regress upon

withdrawal of Eda as in normal skin all placodal cells are committed to producing hair follicles, with none of these cells contributing to the interfollicular epidermis. It is possible that cells lose their commitment to a hair follicle fate and become interfollicular epidermis, though such dedifferentiation is normally observed only upon wounding (Levy *et al.*, 2005). Alternatively, these placode cells may be removed by apoptosis once starved of Eda, or perhaps they survive to find their way into the later forming secondary and tertiary hair follicles.

Dose effects of Ectodysplasin signalling

Though clinical studies have primarily focused on the consequences of complete loss of *EDA* function, it is becoming clear that there is a more graded diversity in the intensity of this signal in human populations. *EDA* mutations that appear to be hypomorphic are associated with non-syndromic tooth agenesis (Li *et al.*, 2008) and even fish tooth development appears to be particularly sensitive to reduced Edar activity (Harris *et al.*, 2008). Conversely, a variant of EDAR with a higher signal transduction potency is associated with the increased hair fibre thickness of East Asian populations (Fujimoto *et al.*, 2008; Mou *et al.*, 2008). Indeed, it is interesting to note that isolated tooth agenesis caused by *EDA* mutation (e.g.(Li *et al.*, 2008) has thus far been reported in Asian families, suggesting that interaction between an *EDA* allele with reduced function and a more potent *EDAR* allele might affect the clinical presentation of HED.

In mouse, too, Eda dose plays a role in determining hair fibre characteristics, with elevated *Eda* expression producing a spiky hair coat due to the angle at which hairs lay relative to the skin. The regulatable Eda model reveals that this characteristic requires a slightly longer signal than that required for primary hair follicle stabilisation. However, removal of Eda prior to birth still confers a shaggy coat texture that appears to be indelible, despite the cyclical nature of hair growth in the adult.

Perhaps the most complex aspect of Eda action relates to its role in shaping individual hair fibres. The majority of hairs in the mouse coat are of the zigzag type. These hairs are produced by the tertiary hair follicles and are bent at constriction sites present at intervals along the fibre. Somewhat paradoxically, loss of *Eda* function or transgenic

Eda expression both have a pronounced straightening effect on these hair fibres. It has been unclear whether this straightening effect is due to alteration of hair follicle identity during development or to a sustained requirement for Eda action in the mature zigzag follicle. The careful microscopic analysis reported by Cui et al. revealed that these straight hairs in Eda transgenic animals do carry regular constrictions, consistent with their possessing a true zigzag identity, but that bends fail to be introduced at these sites. The insertion of constrictions requires continuous Eda expression during fibre growth, but this study (Cui et al., 2009) implies that hair bending is an independent process requiring a very precise location, or perhaps dose, of *Eda* expression to produce the molecular asymmetries responsible for hair shaping (Hammerschmidt and Schlake, 2007).

In all, Cui et al. have shown that Eda action defines appendage structure in a number of ways, acting prior to birth to enable the development of hair follicle types and then to modulate hair thickness and in the adult follicle influencing hair fibre shape. Thus the dose and timing of EDA signalling has significant effects on the external phenotype and genetic tuning of this system is likely to account for some of the extraordinary diversity of cutaneous appendages seen in humans and other vertebrates.

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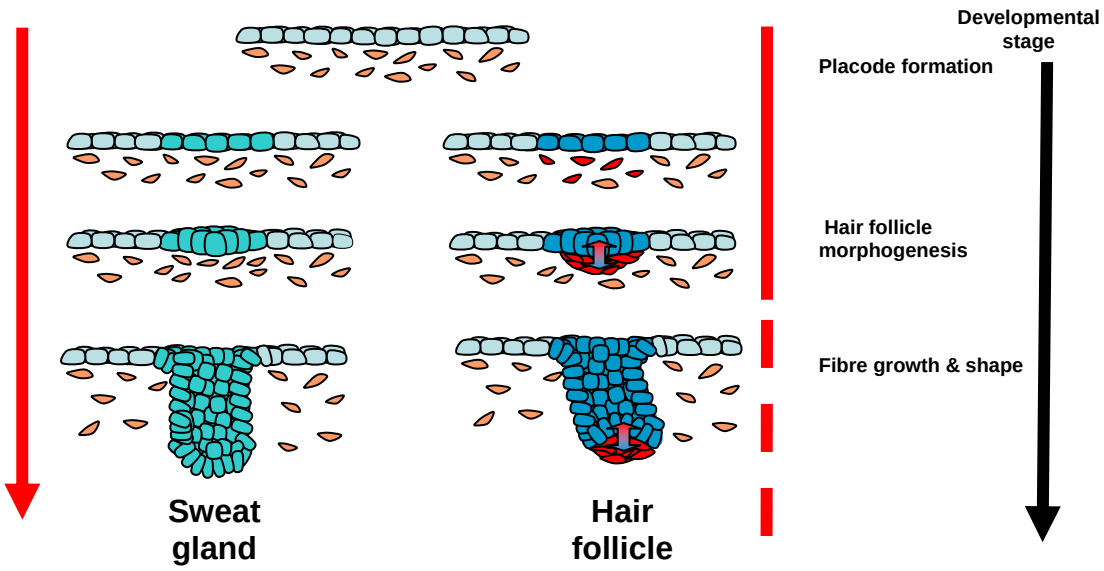
Figure Legend

Figure 1. The influence of Ectodysplasin at different stages of ectodermal appendage development. Acquisition of appendage cell fate is indicated by altered cell shading. Developmental stages with an absolute requirement for Eda to produce a functioning organ are indicated by a solid line, while an Eda influence that is not essential for organ production is indicated by a dashed line. Red/blue block arrows indicate epithelial-mesenchymal interactions in the hair follicle primordium. Formation of a dermal papilla in hair follicle development coincides with independence from Eda action. The stage in sweat gland development at which Eda withdrawal is tolerated is unknown.


Developmental stage
Placode formation
Hair follicle morphogenesis
Fibre growth & shape
Sweat gland
Hair follicle