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The Embryogenesis of the Skin

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1.1 Introduction

The skin is complex tissue and is the biggest constantly renewable organ in the body. It has many different cell types and specialized structures (such as hair, nails, and glands) derived from both embryonic ectoderm and mesoderm. Skin cell types that originate from ectoderm include keratinocytes, neural crest-derived melanocytes, Merkel cells, and neurons, whereas skin cell types that originate from mesoderm include fibroblasts, endothelial cells, adipocytes and bone marrow-derived Langerhans cells [1].

There are two distinct dating systems, one refers to estimated gestational age (EGA) and is used in textbooks and by researchers, in which fertilization occurs on day 1 (first day of the last menstrual period (LMP)), whereas menstrual age, used by obstetricians and most clinicians, has fertilization occurring on day 14 [2, 3].

Fetal skin development can be divided into three temporally overlapping stages: organogenesis (specification), histogenesis (morphogenesis) and maturation (differentiation) [4]. These stages roughly correspond to the embryonic period (0–60 days), the early fetal period (2–5 months), and the late fetal period (5–9 months) of development. The first stage involves the specification of ectoderm lateral to the neural plate to become epidermis and the allocation of subsets of mesenchymal and neural crest cells to become dermis. The second stage is the process by which these committed tissues begin to form their specialized structures, including epidermal stratification, epidermal appendage formation, subdivision between the dermis and subcutis, and vascular formation. The third stage refers to the process by which these newly specialized tissues further differentiate and assume their mature forms [1].

Understanding the stages of normal human skin development allows the definition of critical periods when the skin may be more vulnerable to developmental errors; it provides an opportunity to study the evolution of skin function, establishing a background for understanding the natural history of expression of genetic skin disease in its earliest form; and it provides the essential information for the evaluation of skin samples used in the prenatal diagnosis of genodermatoses for which molecular methods are still not adaptable [5].

The timing at which sampling of the skin for various diagnostic procedures are performed should be recognized. Chorionic villus sampling for fetal DNA is sampled around 10 weeks EGA, amniotic fluid cells (amniocentesis) can be obtained at around

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14–16 weeks EGA, whereas fetal skin biopsy is performed typically at 19–21 weeks EGA [5–8].

1.2 Stages of Skin Development

1.2.1 Embryonic Stage (Specification)

During the third week after fertilization, the human embryo undergoes gastrulation, a complex process of involution and cell redistribution that results in the formation of the three primary embryonic germ layers: ectoderm, mesoderm, and endoderm. Shortly after gastrulation, ectoderm further subdivides into neuroectoderm and presumptive epidermis [1].

1.2.1.1 Epidermis

At six weeks EGA, the epidermis that covers most of the embryo are two-layered epithelium consisting of basal cells and periderm cells [9–11]. The basal cells are more columnar and have intercellular attachment mediated by E- and P-cadherin and they express keratins K5, K14, K8 and K19 [12–14]. The periderm cells are larger and flatter than underlying basal cells and they express K5, K14, K8, K18 and K19 [13–16]. Their apical surfaces are studded with microvilli and tight junctions attach them at their lateral surfaces [11]. Two of the immigrant cells, melanocytes and Langerhans cells, are present in the embryonic epidermis among basal cells. Melanocytes are dendritic as early as 50 days EGA in general body skin but there is no evidence of melanosomes in the cytoplasm [17]. Langerhans cells are recognized in embryonic skin as early as 42 days EGA and they have dendritic morphology and probably derived from yolk sac or fetal liver at this age [18–20]. The third immigrant cells, Merkel cells, can be seen in embryonic palmar skin as early as 55–60 days EGA and they express K8, K18, K10 and K20 [21–25]. K20 is the only keratin found exclusively in Merkel cells. They are distributed randomly and in a suprabasal position. They are neuroendocrine cells acting as slow-adapting mechanoreceptors. It is generally accepted that Merkel cells are derived from keratinocytes in situ [21, 23, 25–27].

1.2.1.2 Dermis

The embryonic dermis is highly cellular and amorphous, consisting of a loose network of mesenchymal cells (fibroblasts, mast cells and skin-derived precursor-SKP-cells) with little intervening fibrous connective tissue matrix, and its origin varies depend on the body site [28]. Dermal mesenchyme of the face and anterior scalp is derived from neural crest ectoderm; the limb and ventral body wall mesenchyme is derived from the lateral plate mesoderm, whereas the dorsal body wall mesenchyme derives from the dermomyotomes of the embryonic somite [29].

The cell migration is promoted through the high water content and hyaluronic-acid-rich environment of the dermal mesenchyme, whereas the exchange of signals between epidermis and dermis is achieved through the compact mesenchyme and is very important in stimulating the onset of appendage formation [5, 30].

Types I, III and VI collagen are distributed uniformly throughout the dermis whereas type V collagen is concentrated primarily along basement membranes and surrounding cells. The ratio of collagen III to collagen I is 3:1, the opposite what it is in the adult [31].

The embryonic dermis does not contain elastic fibers yet, but fibrillin and elastin proteins of the elastic fibers can be identified by immunohistochemistry and microfibrils can be seen by electron microscopy (EM) [11]. Blood vessels have been identified in fetal skin through the process of vasculogenesis (de novo) as early as nine weeks EGA and its pattern varies among different regions of the body [32]. Development of the cutaneous innervation closely parallels that of the vascular system in term of its pattern, rate of maturation, and organization. Cutaneous nerves are composed of somatic sensory fibers, which can be identified at seven weeks

EGA, and sympathetic autonomic fibers, which are not yet recognized [33].

1.2.1.3 Dermoepidermal Junction (DEJ)

The Dermoepidermal Junction (DEJ) connects the developing epidermis (the basal cells) to the dermis and it provides resistance against shearing forces on the skin. The embryonic DEJ is simple, flat, and generic basement membrane and consists of a lamina lucida and a lamina densa. It is composed of molecules that are common to all basement membranes zones (e.g. type IV collagen, laminin, heparin sulfate, proteoglycans, nidogen/entactin) [34]. The α6 and β4 integrin subunits are expressed quite early by embryonic basal cells, but they become localized to the basal surface until after 9.5 weeks EGA, at the same time as bullous pemphigoid antigens (BPA1 and BPA2) are first detected by immunohistochemistry and hemidesmosomes are recognized by EM [34, 35]. The anchoring filaments and anchoring fibrils are identified by nine weeks EGA [4, 36]. Collagen VII (the anchoring fibril protein) is seen slightly earlier at eight weeks EGA [36].

1.2.1.4 Subcutis (Hypodermis)

The subcutis can be first recognized at 50–60 days EGA through a plane of thin-walled vessels that separate the hypodermis from the overlying cellular dermis [4]. However, it is difficult distinguish it from dermis before this time due to similarities in the cells found in both structures and the fact that the adipose tissue is not yet synthesized. There is no morphologic evidence that epidermal appendages started to form in embryonic skin [37].

1.2.2 Embryonic-Fetal Transition (Morphogenesis)

The embryonic-fetal transition is regarded as the most remarkable time in skin development and occurs at around two months (eight weeks) EGA. During this time hematopoiesis has switched from the extraembryonic yolk sac to the bone marrow. The cells of the skin begin to express nearly every characteristic of adult skin. It is also the time that is more vulnerable to errors in development [5].

1.2.2.1 Epidermis

The most important change in the skin at this stage is stratification of the epidermis from two to three cell layers. Intermediate cells are both similar to and distinct from basal and periderm cells. Keratins are more abundant and distributed in a more specific manner than in other two cells. The expression of the major keratin pairs in both basal- and intermediate-layer keratinocytes is now identical to that seen in adult epidermis. The K5 and K14 basal cells keratins are downregulated in the intermediate cells and a new keratin pair, K1 and K10, is synthesized [13, 14]. Also, pemphigus antigen, cornified cell envelope proteins, blood group antigens and cell-surface glycoproteins are expressed in the cytoplasm or on the surface of intermediate-layer cells [38–41]. The cells of both basal and intermediate cell layers express epidermal growth factor (EGF) receptors respond to EGF and retain the ability to proliferate [42–44]. However, only basal cells express P-cadherin, which has a proliferative ability, by the end of the first trimester. Basal cells change in morphology and cell-surface properties after stratification. The cells of the periderm increase in size and begin to develop microvilli-covered blebs that extend from the outermost surface of the cells into the amniotic cavity. Melanocytes are easily recognized in sections of fetal epidermis at eight weeks EGA by their position along the basement membrane, dense cytoplasm, an absence of glycogen and a heterochromatic nucleus [45]. Langerhans cells are more abundant in the epidermis at this stage (~50/mm^2) and they express HLA-DR more than CD1a [46]. Merkel cells are located along the primary epidermal ridges of palmar skin in regular alignment relative to the sites of origin of the sweat duct primordial by the end of the first trimester. Merkel

cells are also present in the dermis of both thick (palms and soles) and hairy skin (around developing hair follicles) where they play a role in attracting and organizing nerve fibers [47]. Epidermal Merkel cells lack nerve growth factor NGF receptors, whereas dermal and follicular Merkel cells are immunopositive [47]. Merkel cells decrease in number during the later stages of fetal development [47].

1.2.2.2 Dermis

Dermal mesenchymal cells still retain glycogen in the cytoplasm, assume a fibroblastic morphology and are responsible for the synthesis of all of the matrix molecules that are characteristic of adult dermis.

There is significantly greater accumulation of small bundles of fibrous proteins within the interstitial space. The papillary and reticular regions of the dermis are demarcated on the basis of increased cell density proximal to the epidermis (the papillary region) and larger collagen fibril diameter and fiber bundle size in the reticular region [31]. The position of the subpapillary vascular plexus of arterioles and postcapillary venules also forms an approximate boundary between these two dermal regions. The dermosubcutaeous vascular plexus demarcates the dermis from the subcutis. Vertically oriented vessels connect the two horizontal plexuses, and fine capillaries extend into the papillary dermis [11]. Nerves are also readily apparent in both regions.

1.2.2.3 Dermoepidermal Junction (DEJ)

The DEJ has acquired all of the adult features that are characteristic for this region. Hemidesmosomes, anchoring filaments and anchoring fibrils are structurally complete and the antigens related to them are also expressed [34–36]. The relative strength of this interface may not be too different from the adult. The dermoepidermal interface is still flat, although modifications of individual basal cells begin to alter the smoothness of this junction.

1.2.2.4 Subcutis

The dermis and hypodermis are distinguished on a morphological basis by differences in the organization and composition of the matrix. The subcutis appears to have markedly fewer cells and smaller bundles of fibrous matrix.

1.2.3 Fetal Stage (Differentiation)

The first stages of fetal skin development occur from the time of the embryonic-fetal transition at two months to the end of the first trimester at three months, when a template of the adult skin is established. During the second trimester, the fetal skin completes the formation of the lanugo hair follicle and synthesis of hair (~17–19 weeks EGA), nail formation (~20–22 weeks EGA) and keratinization of the interfollicular epidermis (~22–24 weeks EGA). During the third trimester, the skin appears structurally similar to postnatal skin, in which the epidermis is fully keratinized, the DEJ starts to have contours, the regions of the dermis are well defined, the adnexae are fully formed and reside deeply in the dermis, and large fat lobules fill the hypodermis [11].

1.2.3.1 Epidermis

The epidermis is now thicker, having more layers of intermediate cells (three by 100–110 days EGA), and the glycogen is still a major constituent of the cytoplasm compared to basal cells. The basal cells having smaller bundles of keratin filaments and the cytoplasm are more ribosome-rich, dense, and organelle-filled. A few layers of thin, flattened, keratinized cells mimicking stratum corneum are visible around 22–24 weeks EGA. With the presence of larger keratohyalin granules and less glycogen content of the cytoplasm, the granular cell layer is now more typical of an adult one. By 22–24 weeks EGA, 1700 Merkel cells/mm^2 can be measured in the epidermis and the number of Langerhans cells begins to increase (~200 cells/mm^2). Melanosomes are transferred to keratinocytes in the fifth month of gestation.

The epidermis is still a less effective barrier than the infant epidermis.

1.2.3.2 Dermis

After embryonic-fetal transition, the presumptive dermis is distinguishable from the underlying skeletal condensations. By 12–15 weeks, the fine interwoven mesh of the papillary dermis can be distinguished from deeper, more fibrillar reticular dermis [4, 31]. Large collagen fibers accumulate in the reticular dermis during the second and third trimester. Definitive elastin fibers first become detectable by EM around 22–24 weeks EGA [48]. By the end of gestation, the dermis is thick and well organized, but is still much thinner than in the adult and has higher water content. Increasing tensile strength and transition from a nonscarring to a scarring response after wounding marks maturation of the dermis. By three months, the distinct horizontal and vertical vascular networks have formed and by the fifth month vasculogenesis has largely ceased and the formation of the complex vascular plexus is initiated by angiogenesis (the budding and migration of endothelium from pre-existing vessels).

1.2.3.3 Dermoepidermal Junction (DEJ)

All the structures of the DEJ were formed in the first trimester, and only a few antigens of the DEJ (AF-1 and AF-2 associated with anchoring fibrils) remain to be recognized at this age [49]. By 19–21 weeks EGA, the hemidesmosomes are present at the basal keratinocytes plasma membrane with adult-like frequency and show a strong association with basal cell keratin filaments. Anchoring filaments and banded anchoring fibrils are well formed [11].

1.2.3.4 Subcutis

The hypodermis remains distinct from the dermis by its less dense matrix and cellularity. Around 15–16 weeks EGA, mesenchymal cells collect in globular arrays surrounded by a capsule-like assembly of matrix. By 18 weeks EGA, lipid droplets are evident within some of the mesenchymal cells, and by 20 weeks fat lobules are established [5].

1.3 Special Features of Developing Human Skin

1.3.1 Periderm

The periderm is the outermost, transient cellular layer of the developing skin of some mammals and birds. The periderm is released as single cells and as sheets of cells into the amniotic fluid at the end of the second trimester when the epidermis keratinizes [11]. The origin of the periderm is unknown, but there are many hypotheses. A reasonable hypothesis would be that the cells of the original, single ectodermal layer of the early embryo divide and give rise to a second cell layer that become superficial to the basal layer. Second, it is also possible that the periderm arises from the cells of the amnion that grow over the single-layered epidermis. A third possibility is that the periderm layer is the original ectodermal layer that covers the very early blastocyst and embryo. Periderm cells do not express the primary K5 and K14 proteins of basal keratinocytes (or the K1/K10 keratins of differentiated keratinocytes) but they do express other keratins in common with the fetal basal cells. The most remarkable features of the periderm are the morphological changes that the periderm cells undergo with progressive stages of development [9]. The periderm cells neither undergo the events of differentiation that are typical for the keratinocytes nor undergo keratinization. The presence of transglutaminase in periderm cells may have implications for the fate of these cells, as the presence of this enzyme is a feature of cells undergoing programmed cell death or apoptosis [50]. The structural properties of periderm cells may provide clues as to the function of the layer. The blebs and microvilli increase the surface area of the periderm as it faces the amniotic fluid, suggesting its role in the

exchange of substances between the fetus and the amniotic fluid. They also have a role in regulating water transport, absorption of nicotine dissolved in amniotic fluid and as a secretory epithelium that adds material to the amniotic fluid [51, 52]. There are no genetic disorders that can affect the periderm cells unlike other cells in the epidermis. Toward the end of the second trimester, they are eventually sloughed and become a component of the vernix caseosa covering the newborn [1].

1.3.1.1 Regionalization in Developing Skin

Regional differences in properties of the skin are well documented in adult skin as well as in developing skin. Without a clear appreciation and accurate knowledge of differences in normal morphology at various sites, structural evaluation of skin samples that may be from unknown regions can be risky.

Also systematic studies of affected fetal skin from multiple regions are valuable to undertake when tissue becomes available, especially in situations where fetal biopsy remains the only current method available to diagnose a severe genetic disease in utero [5].

1.3.1.2 Keratinization

Keratinization of the nails, hair follicles, intraepidermal sweat duct, and the interfollicular epidermis occurs at different times during gestation. The nails are the earliest structures of the skin to keratinize in utero, as early as 11–12 weeks EGA, whereas the follicular epidermis keratinizes consistent with the cephalocaudal direction of follicle morphogenesis [53]. The interfollicular epidermis keratinizes first in thick skin and then in thin skin.

1.3.1.3 Appendage Formation

After the embryonic-fetal transition, around 10–11 weeks EGA, basal epidermal cells undergo proliferation at specifically patterned sites to form buds that grow down into the dermis as hair germs, sweat ducts, or as a nail fold. Nerves and vessels, cellular adhesion molecules (CAMs), soluble mediators and homeobox genes (homeoproteins) have been implicated in the pattern formation of certain appendages. The epithelial-mesenchymal interactions are thought to be responsible for initiation of appendage formation [5].

1.3.1.4 Nail Formation

The distal rays of the digit are evident on the hand of 50-day EGA embryo, and within the next seven days the digit separates. Formation of nail on the dorsal surface of the digits and the eccrine sweat glands on the ventral surface is initiated at about the same time after embryo-fetal transition. By 70 days EGA, proximal, lateral and distal folds establish the boundaries of the nail field externally and sections through the digit reveal a shallow nail fold. By 90 days EGA, the dorsal ridge is evident superficially. The nail fold has invaginated deeply into the dermis and organized into dorsal and ventral layers (the nail matrix) that are distinguished from one another morphologically and functionally. The earliest nail consists of several layers of keratinized cells. By 15 weeks EGA, a thick cornified layer covers the nail bed. This preliminary nail is composed of keratinized epidermal cells from the nail bed rather than from the nail matrix. The nail of a 19-week EGA fetus is established by both the nail matrix and the nail-bed epidermis.

The nail of the newborn is composed of layers derived from the dorsal nail fold (gives outermost layer), the nail matrix (gives the intermediate layer), and the distal nail bed (gives the inner layer).

1.3.1.5 Eccrine Sweat Gland Formation on the Digits

The sweat gland primordial are recognized around 13–14 weeks EGA at regular sites along the epidermal ridges (which first formed around 10–11 weeks EGA) as narrowed, solid, epithelial cords of cells that contain basal cell keratins and express classical carcinoembryonic antigen (CEA) on all

cells [54]. As the cords of epithelial cells elongate into the dermis, a thickening at the terminus defines the glandular segment from the duct. Ductal, secretory, myoepithelial and acrosyringial cell types differentiate in the dermal and intraepidermal regions of the gland and duct [55]. The secretory cells border a central lumen within the gland; myoepithelial cells are evident at the periphery of the structure. Ductal and glandular cells are distinct from one another at 15 weeks EGA by their morphological properties and by differences in expression of keratins (duct) and vimentin (gland) and CAMs [55]. By 22–24 weeks EGA, the sweat glands on the palms and soles have attained the structure of the adult glands, with a coiled secretory segment positioned deeply within the dermis.

1.3.1.6 Eccrine Sweat Gland Formation on the General Body Surface

Eccrine sweat glands form on the general body surface at least four to six weeks later than on the palms and soles.

1.3.1.7 Pilosebaceous Apparatus Formation

It is best described as a composite epithelial-mesenchymal structure. Morphogenesis of the hair follicle begins on the head and face at around 70–80 days EGA, then proceeds in a cephalocaudal direction. The process is completed at around 19–20 weeks EGA. The description of the hair germ, hair peg, bulbous hair peg, and lanugo follicle stages of follicle formation are based on the appearance of vellus hairs on the trunk [5, 11]. Follicles form only during development and decline in numbers as a function of aging. Little is understood about the events that establish the patterns of human follicles or the molecular nature of the inductive messages that start the process. The sites of follicle formation can be recognized even before the hair germs are visible by immunostaining the tissue with an antibody that recognized tenascin [56].

Tenascin is expressed in tissue where there is evidence of matrix remodeling, cell migration, and proliferation. It is also associated with the formation of patterned structures and at sites of epithelial-mesenchymal interaction and mesenchymal condensation [5].

Many signaling molecules including Notch, Sonic hedgehog, Wnt (wingless-related), fibroblast growth factors (FGFs), and bone morphogenetic proteins (BMPs) as well as transcription factors such as HOXC13 AND FOXN1 are important in hair follicle development and cycling [57, 58]. Cells from the basal epidermal layer bud into the dermis at the tenascin-rich sites to become hair germs. Merkel cells, which may play a role in targeting nerve fibers toward the developing appendage, are recognized in some of the developing germs.

At around 13–14 weeks EGA, the hair germs elongate into the dermis as cords of cells called hair pegs. The hair peg consists of an inner core of cuboidal cells (contain intermediate cell keratins) and outer layer of columnar cells (contain same keratin as basal cells) that is associated with the basal lamina. Merkel cells are distributed among the outer root sheath keratinocytes. Early hair pegs are cylindrical, but as they elongate further they develop three regions: a constricted, neck-like connection with the epidermis (the presumptive infundibulum); a central, cylindrical region (the presumptive isthmus); and a terminal zone that becomes widened at its most distal end (the lower follicle and the presumptive bulb). Melanocytes aggregate in the matrix (the bulb) and produce melanin ahead of melanocytes in the general body skin. Between 15 and 17 weeks EGA, bulges of epithelial cells begin to grow out from the epithelial cord on the posterior surface of the follicle. Now the follicle is called a bulbous hair peg. The most superior bulge is the primordium of the sebaceous gland. The second bulge, the "true bulge", is thought to be the site of follicular stem cells and the point of attachment of the arrector pili muscle. A third bulge may form superior to the sebaceous gland as the primordium of the apocrine sweat gland at restricted sites (axilla, areola, scalp, external eyelid, auditory

meatus and anogenital regions) [59]. Merkel cells also concentrate in the bulge at early stages of bulge formation and may play a role in stimulating proliferation or attracting nerve fibers and smooth muscle cells to the site. The first hairs of the fetus are in the anagen phase of the hair cycle. In some newborns, a significant number of them will enter telogen and be shed, creating alopecia that can last up to six months [5].

1.4 Conclusion

In conclusion, the understating of the skin development and embryogenesis is crucial for the diagnosis and management of various congenital or inherited skin diseases. There are many resources available for both diagnosis and treatment of those disorders. Data on genetic test availability are provided by GeneTest (www.genetests.org). Other useful websites include: www.omim.org, www.genedx.com, and www.rarediseases.org. Also, there is online textbook called GeneReviews, containing expert-authored, peer-reviewed disease descriptions ("chapters") presented in a standardized format and focused on clinically relevant and medically actionable information on the diagnosis, management, and genetic counseling of patients and families with specific inherited conditions.

Knowing the mechanisms controlling angiogenesis during intrauterine life holds promise for wound healing and creating new anticancer therapies [58]. Also, knowing the factors involved in wound healing in embryos may point to interventions that may decrease scarring in children and adults. Therefore, advancing the knowledge of skin embryology may lead to life-saving or life-enhancing therapies [58].

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