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Immunoexpression of adhesion molecules during human fetal hair development

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Summary. Introduction. Hair follicles are produced in a cyclical manner and the machinery involved in the reproduction of these follicles is present since the fetal stage. Although extensive research has been done on the human hair follicle, very little is known about the importance of adhesion molecules in its development.

Material and methods. We analyzed here, the immunoexpression of beta-1 integrin, p-cadherin, e-cadherin, and beta-catenin in hair follicles from 26 formalin-fixed and paraffin-embedded skin samples from human embryos and fetus between 12-23 weeks of gestational age.

Results. The adhesion molecules beta-1 integrin and e-cadherin/p-cadherin were expressed from 12 weeks and seemed to play a role in regulating epidermis invagination. Beta-catenin immunostaining was negative in all cases; down regulation of this protein may be necessary for fetal hair development and thus facilitating hair follicle down growth.

Discussion/Conclusion. Adhesion molecules are essential for hair follicle down growth and proliferation; integrins and cadherins play a major role in this process. More studies are needed to describe hair follicle development.

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Introduction

Hair follicles (HFs) are the most dynamic structures in mammals. Their unique structure is in constant renovation and are produced in a cyclical manner. The machinery required for this renovation is present from the fetal stage by virtue of the pluripotent cells located in the bulge HFs (Lavker and Sun, 2000; Moore, 2015). In humans, HFs development occurs early in the fetal period, between 9-12 weeks of pregnancy. HFs distribution starts in the cranial caudal direction, with the primitive follicles first appearing on the face and scalp (Holbrook, 1978; Moore, 2015).

Currently, the process of follicular development and maturation is well understood, but there is lack of knowledge about molecular pathways involved in this process. HFs morphogenesis and subsequent development involve a complex series of events that involve extensive cell and matrix remodeling, and molecules such as proteoglycans and growth factors have been implicated in these processes (Couchman et al., 1990, 1991; Couchman and du Cross, 1995). Signaling between the primitive epithelium and the mesenchyme may be coordinated by skin adhesion molecules; however, the molecular mechanisms of such interactions remain debatable and have generated considerable research interest (Hirai et al., 1989).

Initial research in the field of HFs development focused on identifying the class of adhesion molecules that are differentially expressed in the skin from early development to adulthood (Holbrrok, 1978). Integrins (beta-1 and beta-4) and e- and p-cadherins are important for cell-cell adhesion and for the maintenance of tissue integrity. They are also important for signal transduction in skin development and morphogenesis to form adult and mature tissue (Furukawa et al., 1997). Akiyama et al. (2000) concluded that cell adhesion molecules, including integrins and cadherins, are key regulators of the growth and differentiation of stem cells, for both hair follicle epithelium and epidermal keratinocytes.

The aim of the current study was thus to evaluate the role of adhesion molecules in HFs development. Thus, we selected some adhesion proteins related to clinically important follicular disorders, through review of relevant literature (D'Ovidio et al., 2007; Sprecher, 2016) and studied the expression of these molecules in the early development stages of human fetal hair follicle and their spatiotemporal pattern of expression.

Materials and methods

A retrospective study was conducted by the analysis of hair follicles from 26 Formalin-Fixed and Paraffin-Embedded (FFPE) human embryos and fetus skin samples between 12-23 weeks of gestation, and all specimens were from archives of the Dermatopathology Laboratory, University of São Paulo Medical School. The experiments were performed in accordance with the protocol approved by the Ethical Committee of the institution (protocol number CEP: 0172/08). The regions analyzed included hairy regions from the head and the cases were grouped by the type of hair: terminal hairs from the scalp, supercilium, and vellus hair from the upper lips.

The analysis of beta-1 integrin, e-cadherin, p-cadherin, and beta-catenin protein expression was performed in all samples by immunohistochemistry (IHC). The IHC protocol and details are described in Table 1. We used FFPE fully developed skin specimens as positive controls.

Immunohistochemical technique

The 3 μm slices of each sample were deparaffinized and diaphanized in two xylol baths: the first bath was set at room temperature for 15 minutes and the second bath at room temperature for 10 minutes. Then, the slides were rehydrated in ethanol stream (100%-5 min, 100%-5 min, 95%-2 min, 95%-2 min) and immersed in 10% ammonium hydroxide solution for 10 minutes for the removal of formalin pigments. Afterwards they were washed in running water and then in distilled water. Antigen Retrieval was performed according to the requirement for each primary antibody (Table 1). After washing in running water, distilled water, and Tris HCl washing buffer pH 7.4 for five minutes each, the sections were incubated with selected antibodies overnight.

Subsequently, all specimens were washed with Tris buffer pH 7.4 for 5 minutes. For visualization, all specimens were processed using the EnVision-Permanent Red-Rabbit/mouse kit (Dako), according to the manufacturer's instructions. The development of the chromogenic reaction was monitored in real time under the microscope. All samples were counterstained with Carazzi's Hematoxylin stain for 4 minutes, and all slides were mounted on Permount® resin for light microscopy examination.

Immunohistochemical results analysis

All results were analyzed by two researchers, and the results were photographed and tabulated using Microsoft Excel 2016. Statistical analysis was performed using SPSS v.25.

Results

All specimens were selected from hairy areas. All fetuses were analyzed at the same site (head) and were grouped by type of hair: vellus hair (upper lips) and terminal hair (scalp and supercilium). Mean gestational age was 17.6±3.2 weeks. The results are shown in Table 2.

Integrin expression

Beta-1 integrin was evaluated in all fetuses. Its immunostaining was present in 100% of the 26 specimens analyzed and in all vellus and terminal hairs. It was detected in all follicular regions: outer root sheath region (ORS), inner root sheath (IRS), bulb, isthmus, and primitive epithelium in 100% of cases. Its immunostaining was observed in the follicles at all stages of development, from the placode phase at 12

Table 1. Monoclonal antibodies and protocols used in the study.

Primary anti body	Supplier catalogue number	Company	Antigen retrieval method	Dilution	Incubation time
Beta-1 integrin	NBP2-16974	Novus biologicals	Pressure cooker	1:100	"overnight"
Beta-Catenin	CTNNB1/1509; nbp2-53366	Novus biologicals	Water bath at 98°C for 21 min	1:20	"overnight"
E- cadherin	EP700Y	Cell marque	Pressure cooker	1:100	"overnight"
P- cadherin	GTX113648	Genetex	Pressure cooker	1:100	"overnight"

weeks to the completely developed stage of follicles at 23 weeks. In the placode, immunoexpression was strongest in the lower part and in the primitive follicle, it was also strongest in the lower parts of the ORS. (Figs. 1a/b, 2E/F) (Table 2).

Cadherins complex formation expression

P-cadherin expression

P-cadherin was evaluated in all fetuses. Its immunostaining was present in 76.9% of the 26 specimens analyzed, in 71.4% vellus and 83.3% terminal hairs. Its staining was not only present in the ORS, IRS, and bulb, but also in the primitive epithelium, placodes, and basal areas.

P-cadherin was observed in the follicles at all developmental stages, from 12 weeks in the placode phase and at 23 weeks of development in completely formed follicles. In the placode, its immunoexpression was diffuse in the placode, while in the primitive follicle, it was stronger in the upper and lower parts of the ORS (Figs. 1c/d, 2a/b) (Table 2).

E-cadherin expression

E-cadherin was evaluated in all fetuses. E-cadherin immunostaining was present in 65.4% of the 26 specimens analyzed; 50% in the vellus and 90% in the terminal hairs. E-cadherin was detected in the ORS, IRS, infundibular area and bulb, also in the primitive epithelium.

E-cadherin was observed in the follicles at all developmental stages: from 12 weeks in the placode phase and at 23 weeks of development in completely formed follicles. In the placode, its immunoexpression was stronger in the upper part and diffuse in the primitive follicle. (Figs. 1e/f, 2c/d) (Table 2).

Beta-catenin expression

Twenty-six histological specimens were collected from fetus from 12 to 23 weeks of gestation. The expression of beta-catenin was not found in any of the 26 evaluated specimens; however, expression was found in the positive controls: tonsils and in normal adult scalp (Fig. 1g-i).

Immunohistochemical expression at different phases of hair follicle development

From 12 weeks, in the placode phase, all tested molecules were expressed with an epithelial pattern. P-cadherin was expressed diffusely in the placode area, e-cadherin was expressed in the upper portion of the placode and beta-1 integrin was expressed in the lower portion of the placode. In the hair germ and peg phase, all these molecules showed immunohistochemical expression, and maintained the patterns observed in the next developmental phase.

In the bulbous peg phase, all molecules were expressed: p-cadherin was expressed in the lower and upper portions of the ORS, e-cadherin was expressed in all of the ORS, and beta-1 integrin showed stronger expression in the lower portion of the ORS. A schematic figure of the different phases of hair follicle development is presented in Fig. 2 and Table 2.

Discussion

In this current study, we analyzed the patterns of expression and distribution of adhesion molecules in the development of fetal human HF. In 2008, Lourenço et al. studied these molecules in the fetal epidermis and showed that they were synchronized and regulated in a spatiotemporal manner. Continuing the research started by Lourenço et al. 2008, we chose 26 FFPE skin specimens from human embryos and fetuses because there are interspecies differences in HFs and stem cell markers, which can influence the results in other hosts. The most important differences between humans and other species are the anagen phase duration and asynchronous hair cycle of the human scalp (Oh et al., 2016).

In vitro studies revealed that beta-1 integrin regulates adhesion and differentiation of epidermal cells and plays an essential role in hair germ invagination (Brakebusch et al., 2000). Beta-1 integrin is also responsible for the interactions between ORS and basal layer cells and for the proliferation of follicular cells during their development. It is indispensable for organogenesis and for the morphological and functional integrity of the tissues in adulthood (Brakebusch et al., 1997). Our data show that beta-1 integrin is one of the first molecules expressed in the early phases of HFs development, from

Table 2. Immunoexpression of adhesion molecules in human hair follicle development.

Marker	Immuno expression General (%/ n)	Immuno expression Vellus vs terminal hair	Placode's Localization	Hair germ peg/bulbous peg's Localization
Beta-1 integrin	100% (26)	100% vs 100%	lower part	lower ORS IRS bulb isthmus
E-cadherin	65,4% (17)	50% vs 90%	upper part	ORS IRS Infundibulum bulb
P-cadherin	76,9% (20)	83,3% vs 71,4%	diffuse	lower and upper ORS bulb

ORS, Outer root sheath; IRS, inner root sheath.

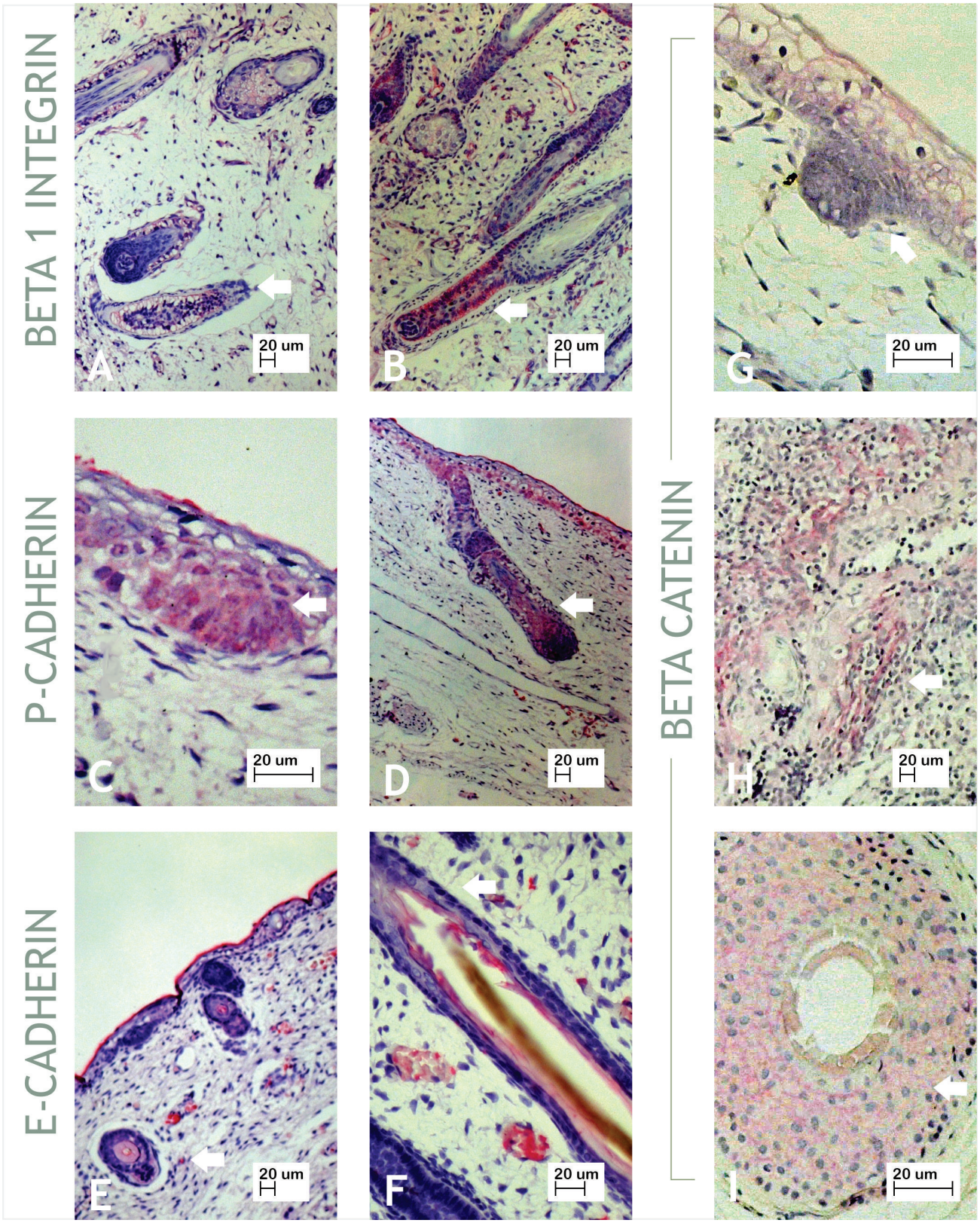

Fig. 1. Immunoexpression of adhesion molecules in fetal primitive hair follicles by immunohistochemistry (IHC). **A.** Beta-1 Integrin expression in a 17-week fetus, in the hair peg. Stronger epithelial expression in the ORS. (white arrows). **B.** Beta-1 Integrin expression in a 20-week fetus in primitive hair follicles. Stronger epithelial expression in the lower parts of the ORS but also in the bulb and primitive epithelium. (white arrows). **C.** P-cadherin epithelial expression in a 12-week fetus, in the placode. (white arrow). **D.** P-cadherin epithelial expression in a 19-week fetus in the hair peg. Stronger expression in the upper and lower ORS parts but some expression also in the primitive epithelium. (white arrow). **E.** E-cadherin epithelial expression in a 15-week fetus hair peg. Stronger expression in the IRS, ORS but also in the primitive epithelium. (white arrows). **F.** E-cadherin epithelial expression in a 23-week fetus in the hair peg. Stronger expression in the IRS and ORS. (white arrows). **G.** Absence of Beta-catenin immunoexpression in 12-week fetal placode. (white arrow). **H.** Beta-catenin positive control in the tonsils (white arrows). **I.** Beta-catenin positive control in adult scalp. (white arrows). A, B, D-F, H, I, x 20; C, G, x 40.

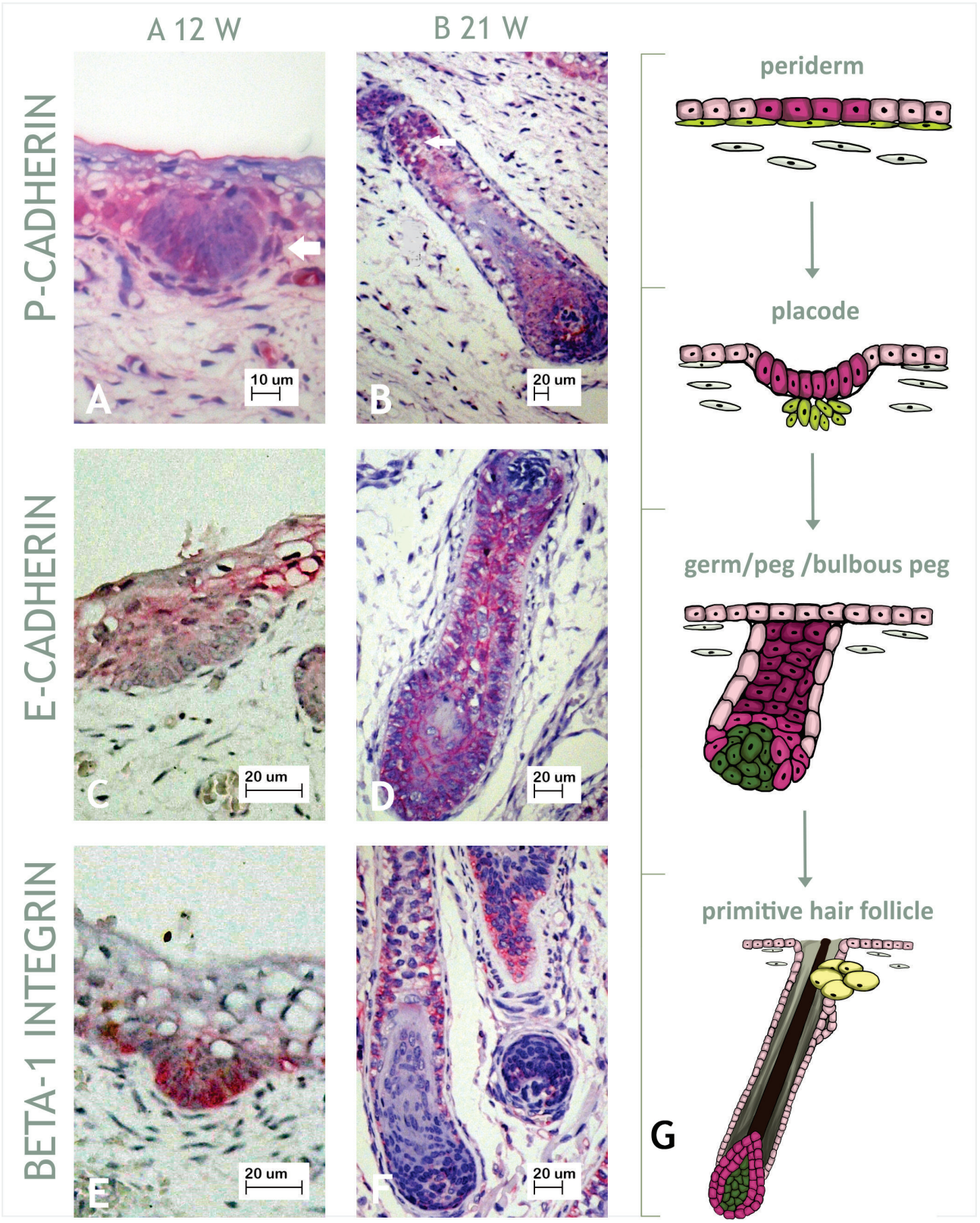

Fig. 2. Evolution of the expression of adhesion molecules in the fetus at different stages of development in the same fetus at 12 weeks and 21 weeks by immunohistochemistry (IHC). Schematic figure of the differents phases of hair follicle development. **A.** Epithelial imunoexpression of 12-week embryo p-cadherin in the placode (diffuse) (white arrows). **B.** Epithelial imunoexpression in the primitive follicle of a 21-week fetus (lower and upper portion of the ORS). (white arrows). **C.** Epithelial imunoexpression of e-cadherin in a 12-week embryo in the placode (upper portion) (white arrows). **D.** Epithelial imunoexpression in the primitive follicle of a 21-week fetus (diffuse). (white arrows). **E.** Epithelial imunoexpression of beta-1 integrin in the placode of a 12-week embryo (lower portion) (white arrows). **F.** Epithelial imunoexpression in the primitive follicle of a 21-week fetus (lower portion). (white arrows). **G.** Schematic figure showing different phases of hair follicle development. The first stage comprises only of the primitive epithelium, which undergoes epithelial invagination to form a hair placoid in the first phase; in the second phase, the epidermal hair germ cells undergo proliferation and polarization, to form the hair peg; finally, these cells form the bulbous peg and the primitive hair follicle. A, C, E, x 40; B, D, F, x 20.

being simply an epidermal projection, its expression became more frequent in human HFs than other adhesion molecules.

In adult skin tissues, integrins are commonly found in the basal layer; however, during the fetal period, their expression is more diffused along the epidermis (Dale et al., 1985). In our cases, we found immunohistochemical expression of beta-1 integrin throughout the skin. These molecules may be targets for treatment of some diseases that have remained untreatable until now. D'Ovidio et al. (2007) showed that there is an altered pattern in the distribution of beta-1 and beta-4 integrins in the lichen planopilaris. A better comprehension of the role of these molecules is important for understanding the normal structure of the skin and its function in either healthy adults or in those with hair follicle diseases.

E-cadherin is an important molecule required for follicle down-growth (Brakebusch et al., 2000). Brakebusch et al. (1997) found that e-cadherin may be seen in the superficial layer of the fetal epithelium and in the development phase of the annexes. In our study, cadherin expression was found in the early phases of follicular development (12 weeks). These data highlight the role of cadherins in the orchestration and signaling of the initial processes of human HFs formation and corroborate with the results of previous studies that indicate the importance of cadherins in vital processes of embryogenesis, formation of tissues and organs, and architectural maintenance (Furukawa et al., 1997; Brakebusch et al., 1997, 2000).

Our results revealed immunohistochemical expression of e-cadherin in an early phase of follicular development in the placode cells and epithelium and in primitive HFs in the ORS, consistent with previous literature. Previously, studies showed that e-cadherin is expressed in all epithelial cells and p-cadherin is expressed more in placode cells, follicular cells and in the hyperproliferative and deep areas of the epidermis. (Lourenço et al., 2008; Sennet et al., 2015; Qiong et al., 2017).

Additionally, we observed that p-cadherin is more specific to HF than e-cadherin. P-cadherin is expressed in HFs, as well as in the whole epithelium; its expression in the HFs was higher than that of e-cadherin in our study. Consistent with the literature, our data showed that e-cadherin immunohistochemical expression was diffused along the primitive epithelium and HFs. P-cadherin in early phases of fetal development has a diffused pattern of expression, but there is a progression during the gestational period, with it tending to be expressed only in the basal layer and HFs.

The immunohistochemical distribution and expression of p-cadherin justifies its crucial role in HFs morphogenesis. It is well known that the loss of function and mutation in the CDH3 gene encoding p-cadherin results in two autosomal recessive allelic diseases: hypotrichosis with juvenile macular dystrophy and hypotrichosis, acrodactyly, and juvenile macular dystrophy. In both syndromes, patients have little hair and progressive loss of vision; in the second syndrome, defects in limb development also occur, such as malformations of the hands and feet (Sprecher, 2016). This evidence highlights the need for more studies on these molecules for their potential applications in the diagnosis of such genetic disorders.

E-cadherin and β-catenin are components of adherent junctions and their down-modulation appears to be a critical event in early morphogenesis and an early step in hair placode morphogenesis and the follicular cycle; the downregulation of these proteins may be a clue for some treatments. Although the relative importance of β-catenin in HF development is known, we could not detect immunoexpression of β-catenin in our study. The down-modulation of β-catenin seems to help the placode down growth and may justify these findings. β-catenin is a central molecule in the Wnt pathway. It is necessary but not enough for hair placode induction during embryogenesis and a selected group of activators and inhibitors of different pathways operate in parallel to establish correct patterning of hair follicles (Krause and Foitzik, 2006; Chen et al., 2012; Tsai et al., 2014; Ahmed et al., 2017). Wnt/β-catenin signaling markedly increases during anagen and subsequently decreases as the hair follicle enters catagen and telogen. This transition is important for catagen induction, including quiescence and maintenance of hair follicle stem cells in telogen. Epidermal growth factor receptor (EGFR) is a molecule that correlates to hair follicle morphogenesis and is implicated in the hair follicle cycle due to the correlation of its expression with the Wnt/ β-catenin pathway. EGFR is required for anagen to catagen transition by inhibiting Wnt expression and β-catenin activity in hair follicles before the onset of catagen.

Many studies that have analyzed β-catenin in hair follicle development have been performed in animals. In these studies, β-catenin was expressed in the ORS, IRS, matrix, and hair shaft and in the HFs epithelial region (Lin et al., 2015). The literature shows β-catenin correlation with CD133, a protein found only in humans. This protein was correlated with the loss of membrane β-catenin in the regions where they were expressed. We speculate that in our study, CD133 and EGFR were overexpressed and β-catenin was not expressed. Thus, our next study will analyze CD133 and EGFR expression, to better understand why β-catenin was not expressed in our cases (Gay et al., 2015; Tripurani et al., 2018).

Our data support those of previous studies, which indicated that adhesion molecules are essential for HF down growth and proliferation. Beta-1 integrin and e-cadherin/p-cadherin play a major role in this process. Terminal hairs had stronger expression than vellus hairs on those molecules. Down-regulation of beta-catenin seems to be useful for follicle down growth. Further advanced investigations are needed to understand human HFs development in detail to help HFs disease pathogenesis, diagnosis, and treatment.

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Conflict of Interest. The authors declare that they have no conflicts of interest.

References

Ahmed N., Ghatak S., El Masry M., Gnyawai S., Roy S., Amer M., Everts H., Sem C. and Khanna S. (2017). Epidermal E-cadherin dependent β-catenin pathway is phytochemical inducible and accelerates anagen hair cycling. Mol. Ther. 25, 2502-2512.

Akiyama M., Smith L. and Shimizu H. (2000). Changing patterns of localization of putative stem cells in developing human hair follicles. J. Invest. Dermatol. 114, 321-327.

Brakebusch C., Hirsch E., Potocnik A. and Fassler R. (1997). Genetic analysis of beta1 integrin function: confirmed, new and revised roles for a crucial family of cell adhesion molecules. J. Cell Sci. 110, 2895-2904.

Brakebusch C., Grose R., Quondamatteo F., Ramirez A., Jorcano J. and Pirro A. (2000). Skin and hair follicle integrity is crucially dependent on beta 1 integrin expression on keratinocytes. EMBO J. 19, 3990-4003.

Chen D., Jarrell A., Guo C., Lang R. and Atit R. (2012). Dermal beta-catenin activity in response to epidermal Wnt ligands is required for fibroblast proliferation and hair follicle initiation. Development 139, 1522-1533.

Couchman J. and du Cros D.L. (1995). Proteoglycans and associated proteins of the mammalian hair follicle. J. Invest. Dermatol. 104, 40S-1S.

Couchman J., King L. and McCarthy J. (1990). Distribution of two basement membrane proteoglycans through hair follicle development and the hair growth cycle in the rat. J. Invest. Dermatol. 94, 65-70.

Couchman J., McCarthy K.and Woods A. (1991). Proteoglycans and glycoproteins in hair follicle development and cycling. Ann. NY Acad. Sci. 642, 243-251.

D'Ovidio R., Sgarra C., Conserva A., Angelotti U., Erriquez R. and Foti C. (2007). Alterated integrin expression in lichen planopilaris. Head Face Med. 3, 11.

Dale B., Holbrook K., Kimball J., Hoff M. and Sun T. (1985). Expression of epidermal keratins and filaggrin during human fetal skin development. J. Cell Biol. 101, 1257-1269.

du Cros D., LeBaron R. and Couchman J. (1995). Association of versican with dermal matrices and its potential role in hair follicle development and cycling. J. Invest. Dermatol. 105, 426-431.

Furukawa F., Fujii K., Horiguchi Y., Matsuyoshi N., Fujita M., Toda K., Imamura S., Wakita H. and Shirahama S. (1997). Roles of E- and P-cadherin in the human skin. Microsc. Res. Tech. 38, 343-352.

Gay D., Yang C., Plikus M., Ito M., Rivera C., Treffeisen E., Doherty L., Spata M., Millar S. and Cotsarelis G. (2015). CD133 expression correlates with membrane beta-catenin and E-cadherin loss from human hair follicle placodes during morphogenesis. J. Invest. Dermatol. 135, 45-55.

Hirai Y., Nose A., Kobayashi S. and Takeichi M. (1989). Expression and role of E- and P-cadherin adhesion molecules in embryonic histogenesis. I. Lung epithelial morphogenesis. Development 105, 263-270.

Holbrook A. (1978). Structure of the human fetal hair canal and initial hair eruption. J. Invest. Dermatol. 71, 385-90.

Krause K. and Foitzik K. (2006). Biology of the hair follicle: The basics. Semin. Cutan. Med. Surg. 25, 2-10.

Lavker M. and Sun T. (2000). Epidermal stem cells: properties, markers, and location. Proc. Natl. Acad. Sci. USA 97, 13473-13475.

Lin C., Yuan Y., Chen X., Li H., Cai B., Liu Y. and Huang K. (2015). Expression of Wnt/β-catenin signaling, stem-cell markers and proliferating cell markers in rat whisker hair follicles. J. Mol. Histol. 46, 233-240.

Lourenco S., Kamibeppu L., Fernandes J., Sotto M. and Nico M. (2008). Relationship of adhesion molecules expression with epithelial differentiation markers during fetal skin development. J. Cutan. Pathol. 35, 731-737.

Moore L. (2015). The developing human: clinically oriented embryology Sauders Philadelfia. 451-455.

Oh J., Kloepper J., Langan E., Kim Y., Yeo J., Kim M., His T., Rose C., Yoo G., Lee S., Seykora J., Kim J., Sung Y., Kim M. , Paus R. and Plinkus M. (2016). A guide to studying human hair follicle cycling *in vivo*. J. Invest. Dermatol. 136, 34-44.

Qiong S., Weirong Y., Yong F., Min Y. and Penggao Y. (2017). Beta-catenin can induce hair follicle stem cell differentiation into transit-amplifying cells through c-myc activation, Tissue Cell 49, 28-34.

Sennett R., Wang Z., Rezza A., Grisanti L., Roitershtein N., Sicchio C., Mok K., Heitman N., Clavel C., Ma'ayan A. and Rendi M. (2015). An integrated transcriptome atlas of embryonic hair follicle progenitors, their niche, and the developing skin. Dev. Cell 34, 577-591.

Sprecher E. (2016). Inherited hair disorders. In: Rook's Textbook of Dermatology. Ninth Edition. Griffiths C.E., Barker J., Bleiker T., Chalmers R. and Creamer D. (eds). Vol 3, part 8, 89.1

Tripurani S.K., Wang Y., Fan Y.X., Rahimi M., Wong L., Lee M.H., Starost M.F., Rubin J.S. and Johnson G.R. (2018). Suppression of Wnt/β-catenin signaling by EGF receptor is required for hair follicle development. Mol. Biol. Cell 1, 2784-2799.

Tsai S., Sennett R., Rezza A., Clavel C., Grisanti L., Zemla R., Najam S. and Rendl M. (2014). Wnt/β-catenin signaling in dermal condensates is required for hair follicle formation. Dev. Biol. 385, 179-188.

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