MODULATION OF EXPRESSION OF 55- AND 40-KILODALTON PREKERATINS OF SIMPLE EPITHELIUM DURING SKIN MORPHOGENESIS IN RATS

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The prekeratins (PC) are a family of about 20 related proteins which are components of the intermediate filaments (IF) of epithelial cells [6]. Different morphological types of epithelium contain different sets of PC [3, 6]. To explain the relationship between composition of IF and morphological structure, it is necessary to study expression of individual PC during normal ontogeny. The most interesting tissues are naturally those in which epithelium of one morphological kind changes into another. One such example is the ectoderm of the skin in which, during ontogeny, the epithelium changes from the simple to the stratified type of organization. In addition. some cells of the ectoderm migrate into the mesenchyme, where they form hair follicles and other appendages of the skin [4, 5]. This diversity of the morphogenetic processes taking place during development of the skin provides a wealth of material with which to study the time course of PC expression.

The writers previously obtained monoclonal antibodies against individual PC of rat simple epithelium with mol. wt. of 55 (PC55) and 40 kilodaltons (PC40) [1]. According to the data in the literature [6], these PC correspond to human PC Nos. 8 and 19. By immunofluorescence staining of sections of embryos and of the skin in the postnatal period of development of rats with these antibodies it was possible to trace changes in expression of the corresponding PC. The results showed that the simple ectoderm which precedes the epidermis contains PC55 and PC40 — the PC of the simple epithelium of adult animals. Transient expression of these same PC is found in cells forming hair follicles.

EXPERIMENTAL METHOD

Whole Fisher rat embryos of both sexes, aged 12 days, segments of the uterus containing embryos from 8 to 11 days of development, and pieces of skin from different parts of the body of newborn or adult rats were mounted in gelatin (7% in isotonic phosphate buffer, pH 7.2) and frozen in liquid nitrogen. Day 0 of pregnancy was identifed by the presence of spermatozoa in vaginal smears. Frozen sections 6-8 μ thick were cut for immunofluorescence and histological investigations. For indirect immunofluorescence staining monoclonal antibodies of clones E2 and E3, against PC55 and PC40, respectively, were used [1] . In some cases, to reveal the basement membrane, the sections were stained simultaneously with rabbit antiserum against laminin (generously provided by A. V. Lyubimov, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). Commercial antisera were used as the second antibodies: fluorescein-labeled donkey serum against mouse immunoglobulins (Sigma, USA) and rhodamine-labeled porcine serum against rabbit immunoglobulins (Dako, Denmark).

EXPERIMENTAL RESULTS

In the early stages, from the 8th to the 14th days of embryonic development, the future rat epidermis consists of a sheet of undifferentiated cuboidal cells, arranged in one or two layers (Fig. 1a), known as the "integumentary ectoderm" [3, 4]. Indirect immunofluorescence with antibodies of clones E2 and E3 showed that PC55 is only weakly expressed at these stages by all cells of this epithelium (Fig. 1b), whereas PC40 is absent. On the 11th and 12th days the integumentary ectoderm of the embryo divides into two zones, which differ in their con-

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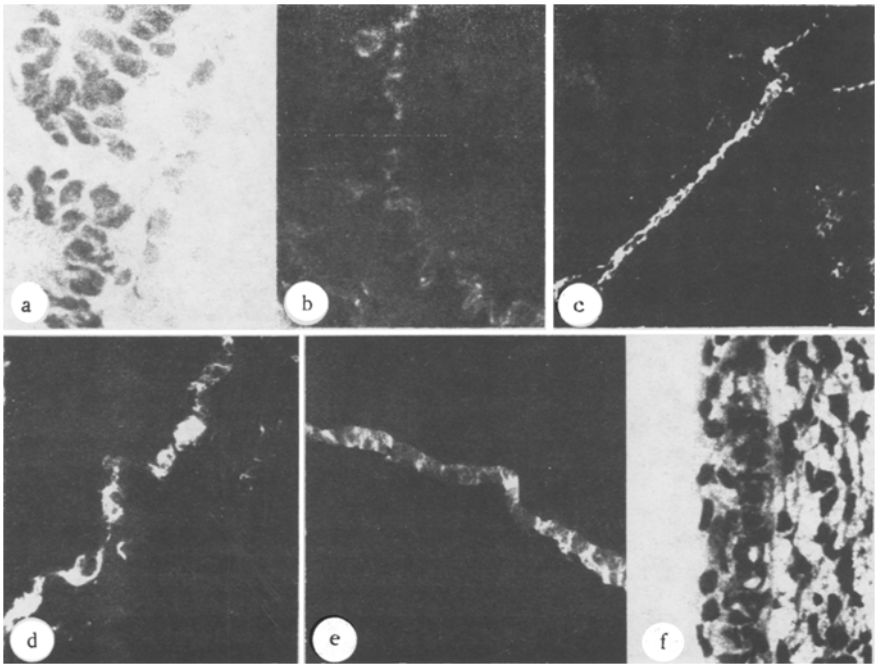

Fig. 1. Integumentary ectoderm of 10-15-day rat embryos. a, b) Dorsal ectoderm of 10-day embryo: sheet of cells consisting of a single layer, containing a little PC55 (300 ×); c, d) ectoderm of ventral aspect of 12-day embryo is brightly stained (c, 60 ×; d, 300 ×); e) dorsal ectoderm of 14-day embryo (400 ×); f) dorsal epidermis of 17-day embryo. Three layers are clearly visible: periderm (top layer of large cells), intermediate, and basal (400 ×). L) Liver, H) heart. a, f) Hematoxylin and eosin; b, c, e) indirect immunofluorescence with E2 antibodies against PC55; d) with E3 antibodies against PC40.

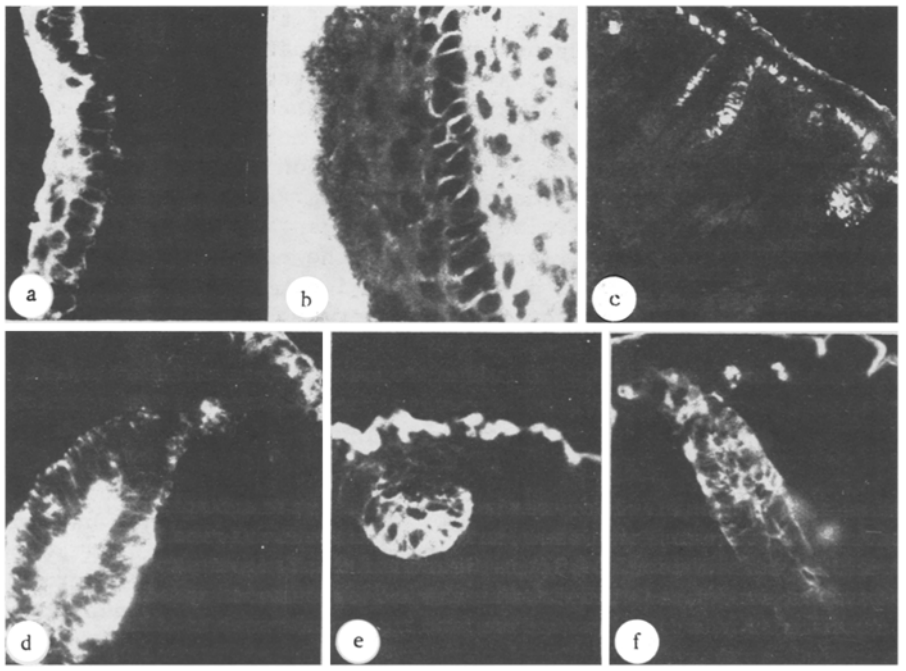

Fig. 2. Skin of 15-20-day embryos. a) Dorsal epidermis of 16-day embryo: cells of periderm are brightly stained (350 ×); b) epidermis of 20-day embryo: stratum basale, stratum spinosum, and stratum corneum are clearly visible (350 ×); c) epidermis of snout of 20-day embryo. Single cells of of stratum basale and of outer root sheath contain PC55; the latter are marked by arrows (150 ×); d) newly formed follicle of vibrassae of 17-day embryo (350 ×); e, f) 20-day embryo: different stages of formation of hair follicles (300 ×). b) Hematoxylin and eosin; a, c-f) indirect immunofluorescence with E2 antiboides against PC55.

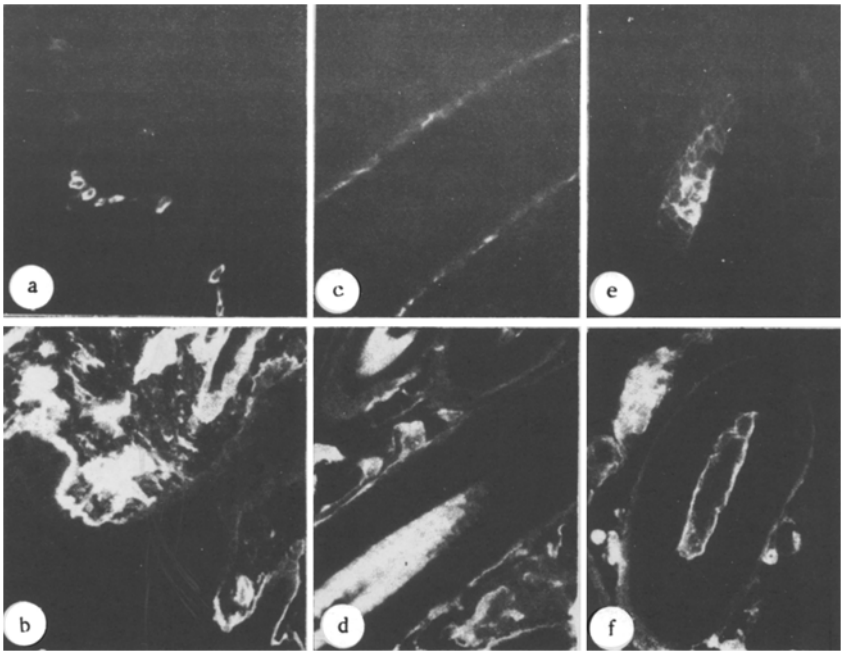

Fig. 3. Skin of newborn animals (15 days after birth). a, b) Epidermis: single cells containing PC55 can be seen near the mouth of follicles (arrow); c, d) cells of stratum basale of outer root sheath contain PC55 (d — growing hair is nonspecifically stained by rhodamine); e, f) matrix of hair follicle (diagonal section) with a group of cells containing PC55. Double indirect immunofluorescence with antibodies against PC55 (a, c, e) and against laminin (b, d, f). 300 ×.

tent of PC40 and PC55. The ventral aspect of the embryo, in the region of the heart, at this stage of development is covered with ectodermal cells which stain intensely for both PC55 (Fig. 1c) and PC40 (Fig. 1d). Fluorescence of the ectoderm of the remainder of the body remained the same as in earlier embryos: weakly positive on staining for PC55 (Fig. 1e) and negative on staining for PC40. This distribution of PC persisted in the ectoderm until 15 days, i.e., until the beginning of formation of a stratified sheet consisting of cell layers at different stages of differentiation: outer (the periderm), intermediate, and basal (Fig. 1f). At this time of embryonic development a high concentration of the two PC was observed in the periderm: PC55 in the periderm of the whole embryo (Fig. 2a), and PC40 also in the periderm in the region of the heart. In addition, a small quantity of PC55 was found in cells of the stratum basale. This PC completely disappeard from the majority of cells of the stratum basale of the ectoderm by the 20th day of development, i.e., by the time when the structure of the tissue became characteristic of the adult epidermis: the periderm disappeared partially or completely and clearly distinguishable stratum spinosum and stratum corneum were formed (Fig. 2b). At this stage of development E2 antibodies revealed only a few single cells containing PC55 in the skin, where they were arranged in the stratum basale of the epidermis. These cells were distributed singly or in small groups. They were most numerous in the epidermis of the snout (Fig. 2c). By means of an immunoelectron-microscopic investigation Moll et al., showed that solitary cells containing PC of simple epithelia and located in the stratum basale of human fetal skin are Merkel's cells [8]. However, since they had antibodies only against PC No. 18, Moll et al., were compelled to identify the remaining PC biochemically. This made it difficult to study small cell populations in the developing epidermis. Since we used antibodies against PC55 we were able to discover that this PC is present in the cells of hair follicles in the early stages of their formation.

Primitive follicles of the vibrissae are the first to appear, at about the 14th-15th days of embryonic development of the rat, by penetration of epidermal bands into the underlying connective tissue. In the first 2 days all the cells of this epidermal band stain intensely with antibodies against PC55 (Fig. 2d). Later, however, the number of positive cells gradually decreases. At the 20th day only some of the cells of the outer hair root sheath still contain PC55 (Fig, 2c). Primitive hair follicles in the remaining parts of the body are formed in a similar manner, but later — starting with the 18th day. Since at this time the basal cells of the epidermis have already lost virtually all their PC55, it will be clear that this protein

appears in the earliest stages of follicle formation (Fig. 2e, f). Possibly some single cells of the stratum basale containing PC55 in the period between the 18th and 22nd days of embryogenesis are future hair follicles.

No cells staining positively with antibodies against PC40 were present in the epidermis and its derivatives in rats after birth. On staining for PC55 on the 1st day of postnatal development, single positive cells were found in the stratum basale of the epidermis. These cells were often located near the mouth of certain hair follicles (Fig. 3a, b). A difficult picture to interpret also was observed in the hair follicles of the postnatal animals. In some of them, in the stage of hair growth (anagen), cells of the outer root sheath contained PC55 (Fig. 3c, d). Other hair follicles, as a rule of smaller diameter but, in all probability, at this same stage of the hair cycle, contained PC55 in a small group of cells of the hair matrix, arranged asymmetrically relative to the connective-tissue papilla of the follicle (Fig. 3e, f). Further investigations are needed in order to obtain a complete explanation of the principles of PC55 expression in hair follicle cells of adult rats.

During the development of the stratified keratinizing epidermis of the skin in rats from the simple integumentary ectoderm, the composition of the PC in the cells participating in this process thus changes at least 3 times. First, in the ectoderm of the cardiac prominence of the 11-12-day embryo, PC40 appears and there is a sharp increase of PC55 expression. Second, during the formation of the stratified epidermis, PC55 and PC40 gradually disappear. Third and last, in the early stages of hair follicle formation PC55 appears, whereas later, after the formation of the mature follicle, this protein disappears from most cells.

These last two events are closely connected with certain morphogenetic processes. The appearance of the differentiated stratified epithelium instead of the undifferenctiated epithelium takes place parallel with replacement of PC of the simple epithelium by PC of the stratified keratinizing epithelium. Migration of epidermal cells into the dermis during follicle formation leads to re-expression of PC55 and, as Moll et al., showed previously [7], to inhibition of some keratins of stratified keratinizing epithelium. After the mature structure of the follicle has been formed, the reverse process takes place in most cells. Recent data suggest that the synthesis of the different types of PC is controlled by intercellular junctions of epithelial cells [2]. In all the cases which we examined, reorganization of intercellular junctions may have led to the corresponding changes in PC sets. The appearance of PC40 and increased expression of PC55 in the ectoderm in the region of the heart can be explained by reorganization of intercellular junctions in that zone. When the heart begins to work the ectodermal membrane of the cardiac prominance may perhaps need strengthening. It can be tentatively suggested that this happens through the appearance of new intercellular junctions, which lead to expression of PC40 and to an increase in the expression of PC55.

With respect to differentiation of the integumentary ectoderm into the epidermis we thus see the phenomenologic connection between expression of PC of the simple epithelium and certain morphogenetic processes. The role of this switch of PC in the mechanisms of these processes is not yet clear. Simplified systems of morphogenesis in vitro or ability to modify the spectrum of synthesized PC at will are necessary for the elucidation of this process.

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