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Letter to the Editor

Caspase-14 is expressed in the epidermis, the choroid plexus, the retinal pigment epithelium and thymic Hassall's bodies

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Dear Editor,

Caspases are cysteine proteases that cleave their substrates after an aspartate residue. So far, 13 members of this family have been described in mammalia and most of them are known to play a role in apoptosis or inflammation.[1,2] The involvement of caspases in processes such as lens epithelium differentiation and erythropoiesis has also been reported. We previously identified caspase-14 as a short prodomain caspase that is processed during normal epidermal differentiation without the requirement of any apoptotic or inflammatory stimuli to activate other caspases.[3,4] Moreover, caspase-14 was shown to remain unprocessed under apoptotic conditions, indicating that it does not participate in the classical apoptotic pathway.[4]

We and other research groups have reported the caspase-14 expression pattern in the epidermis.[4–7] Kuechle *et al* reported that during mouse embryonic development caspase-14 is only present in the epidermis.[7] However, we found that the expression of caspase-14 is not exclusively limited to the epidermis. To further examine the expression pattern of caspase-14, we applied immunohistochemistry on paraffin sections of BALB/c embryos as well as on adult mouse and adult human tissues using a caspase-14-specific antibody. Compared to the other ubiquitously expressed caspases described,[1,8] the expression pattern of caspase-14 is very restricted. Protein expression could be detected in the differentiating keratinocytes of the epidermis, the epithelial cells of the choroid plexus, the pigmented layer of the retina and Hassall's bodies in the thymus, both in embryonic and adult tissues (Figure 1A). This restricted expression of a caspase is quite unique. Remarkably, those tissues in which caspase-14 is expressed have a function involving barrier formation.

The skin is a first line of defence against different environmental factors to which an organism is exposed. This epidermal protective barrier is established by keratinocytes that undergo a complex program of terminal differentiation. The primary keratinocytes of the *stratum basale* will differentiate and keratinize towards the surface of the skin. During this process of cornification, the cells are tightly connected by desmosomes. Epidermal expression of caspase-14 is restricted to the differentiated keratinocytes in the suprabasal layers, both in the embryo (Figure 1a, a) and in adult mouse and human skin (Figure 1A, d),[4] while it is not present in the basal layer (Figure 1A, a, d) or the embryonic periderm (Figure 1A, a).[4] In the inner and outer root sheet of the hair follicle and in the sebaceous gland, caspase-14 is expressed as well (Figure 1A, g and h). In these epidermal derivatives, the keratinocytes undergo a program that leads to cellular demise: the outer root sheet is continuous with the epidermis, the inner root sheet cells undergo regular desquamation, while the cells of the sebaceous gland are lost during holocrine secretion. Sweat glands, consisting of 'viable' keratinocytes performing eccrine secretion, do not contain any caspase-14.[4] In the epidermis, caspase-14 is present in both the cytoplasm and the nucleus (Figure 1A, a, d, g, h). Caspase-14 is found in all suprabasal layers of normal epidermis, but not in the parakeratotic plaques of psoriasis patients in which the barrier function has been disrupted.[4]

The ventricles of the mammalian brain are lined by a monolayered epithelium, viz. the ependyma. In contrast to the ependymal layer, the epithelial cells of the choroid plexus are sealed together by tight junctions. These impede diffusion of molecules between the blood and the cerebrospinal fluid, and as such constitute the blood–brain barrier. Caspase-14 protein expression was detected in the cytoplasm of the choroid plexus epithelial cells, while it is absent in the cells of the ependymal layer, both in embryonic (Figure 1A, b), adult mouse (Figure 1A, e) and human samples (Figure 1B, a). The presence of caspase-14 mRNA in the brain was reported previously; in an experimental pneumococcal meningitis model, caspase-14 mRNA levels were shown to have increased in the brain.[6,9]

Choroid plexus tumours constitute 2–4% of brain tumours in children.[10] They give rise to symptoms such as vomiting, strabism, headache and even hydrocephalus because of an increased intracranial pressure. In contrast to normal human choroid plexus tissue, caspase-14 expression was absent in samples of three different patients suffering from a choroid plexus papilloma (Figure 1B).

The retina lines the inside wall at the back of the eye and is composed of an inner thicker layer called the sensory retina and an outer layer named the retinal pigment epithelium (RPE), which is thought to be involved in the maintenance of the rods and cones of the sensory retina.[11] The choroid is the layer behind the retina containing the blood vessels that nourish the rods and cones. Initially, the intraretinal space separates the sensory retina and the RPE, but during development both layers will make contact so that the intraretinal space disappears. In the embryo, caspase-14 is present in the RPE when it makes contact with the sensory retina, but not when both layers are separated by the intraretinal space (Figure 1A, c). A similar expression was

observed in the eye of adult BALB/c mice (data not shown). This suggests that molecules involved in cellular contact may regulate caspase-14 expression in this tissue.

Using RT-PCR, it was shown that caspase-14 mRNA is present in the thymus.[6] The medulla of the thymus comprises different cell types of variable origin. Caspase-14 protein was detected in Hassall's bodies (i.e. concentrically lamellated, rounded and keratinizing cell structures) in adult mouse and human thymus (Figure 1A, f, i). It is not clear whether these structures are of ectodermal or endodermal origin. Hassall's bodies reside in the medulla of the thymus that developed from the third and fourth endodermal pharyngeal pouches. However, Hassall's bodies are believed to develop from cells of neural crest origin.[12] Although both the function and origin remains unknown, several analogies between Hassall's bodies and the epidermis have been observed. Keratinocytes of the epidermis and Hassall's bodies have similar antigenic properties, since they both express typical markers for keratinocyte terminal differentiation, such as keratins and fillagrin.[13,14] In this respect, it is not completely surprising that caspase-14 expression is also found in the Hassall's corpuscles of the thymus.

Caspases are expressed as prozymes, containing an N-terminal prodomain, a large subunit with an approximate size of 20 kDa (p20) and a small subunit of about 10 kDa (p10).[1] For both murine and human caspase-14, a splice variant has been described.[5,7] The mouse caspase-14B splice variant lacks the sequence corresponding to exons 5 and 6, and thus gives rise to a form almost completely lacking the p10 subunit. This would generate a nonactive form of caspase-14. Alternative splicing of human caspase-14 results in a frame shift, eventually leading to human caspase-14B whose C-terminal sequence no longer shows similarity to a typical caspase p10 sequence. In order to investigate which caspase-14 splice variant is expressed in the tissues described above, mRNA was isolated from different mouse tissues and used for RT-PCR. A band of 795 bp was detected that corresponds to mouse caspase-14A, but only in the skin and eye an additional band of 541 bp was found that correlates to mouse caspase-14B (Figure 1C).

The human caspase-14B splice variant was amplified and cloned using RNA isolated from differentiated HaCaT cells. Upon overexpression of human caspase-14A and human caspase-14B in HEK293T cells, lysates were prepared and used for Western analysis (Figure 1D). Comparison to lysates of normal human skin revealed that the caspase-14B protein cannot be detected by means of Western blotting analysis. Therefore, we suggest that caspase-14B is probably not implicated in normal skin differentiation.

In contrast to an earlier paper reporting that caspase-14 is exclusively expressed in epidermal tissue,[7] we show here that caspase-14 is expressed in all suprabasal layers of the

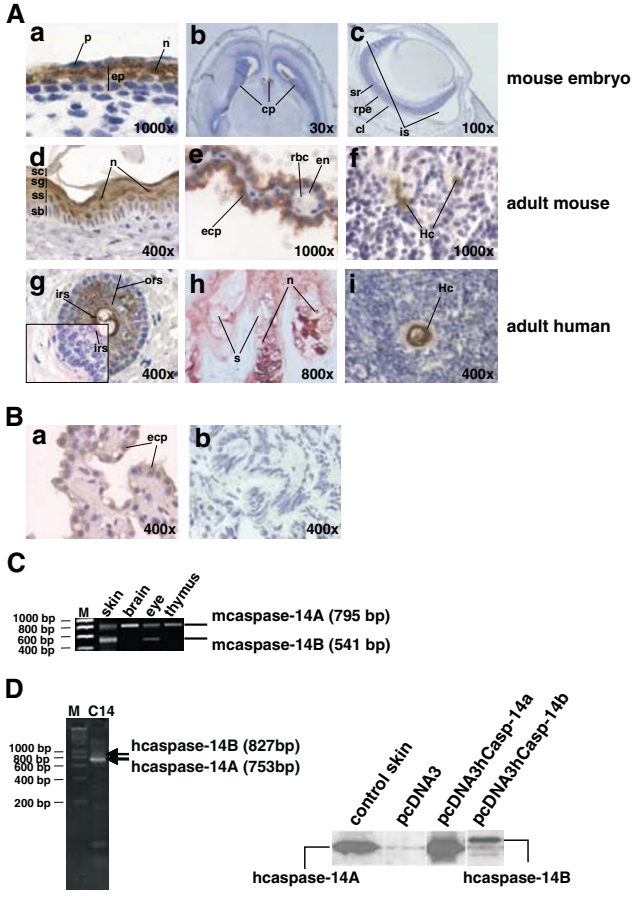

Figure 1 (**A**) Caspase-14 protein expression pattern. Paraffin sections of 15.5 days postcoitum BALB/c mouse embryos (**a–c**), adult BALB/c mouse (**d–f**) and adult human tissues (**g–i**) were used for immunohistochemical analysis with an caspase-14 antibody as described previously.[4] (**a**) section through BALB/c embryonic skin. (**b**) BALB/c embryonic brain. (**c**) BALB/c embryonic eye and inset at higher magnification. (**d**) adult murine skin. (**e**) murine choroid plexus. (**f**) Hassall's corpuscle in murine thymus. (**g**) human hair follicle and inset with hematoxylin/eosin staining of section through the same hair follicle. The cells of the inner root sheet can be recognised as flattened cells with strong eosinophilic staining that cover the hair shaft. (**h**) human sebaceous gland. (**i**) Hassall's corpuscle in human thymus. (cl: choroid layer, cp: choroid plexus, ecp: epithelial cells of the choroid plexus, en: endothelium, ep: epidermis, Hc: Hassall's corpuscle, irs: inner root sheet, is: intraretinal space, n: nucleus, ors: outer root sheet, p: periderm, rbc: red blood cell, rpe: retinal pigment epithelium, s: sebum, sb: *stratum basale*, sc: *stratum corneum*, sg: *stratum granulosum*, sr: sensory retina, ss: *stratum spinosum*). (**B**) Caspase-14 protein expression is down-regulated in choroid plexus papilloma. Paraffin sections of normal human choroid plexus (**a**) and choroid plexus papilloma tissue (**b**) were analysed for caspase-14 protein expression by immunochemical staining as described previously.[4] Plexus choroideus papillomas are described as cauliflower-like masses, consisting of connective tissue fronds covered by a single layer of cuboidal to columnar epithelial cells with round or oval basally situated nuclei (ecp: epithelial cells of the choroid plexus). (**C**) Murine caspase-14A is expressed in skin, brain, eye and thymus. Expression of caspase-14A and -B in murine skin, brain, eye and thymus was analysed by means of RT-PCR. Following primers were used as forward primer and reverse primer respectively (the sequence matching the murine caspase-14 sequence is underlined): 5′-CCCAAGCTTCCACCATGGAGTCA-GAGATGAGTGA-3′ and 5′-GGAATTCTTATTGCAAATAGAGCTTCTTCC-3′. (**D**) Human caspase-14B is not expressed in normal epidermis. RNA isolated from differentiated HaCaT cells was used for cDNA synthesis. Both caspase-14A and -B could be amplified by means of RT-PCR using primers that correspond to the 5′ and 3′ end of the ORF of human caspase-14 (the sequence corresponding to hcaspase-14 is underlined: forward 5′-ACCCAAGCTTCCACCATGAG-CAATCCGCGGTCTTTG-3′ and 5′-CCGCTCGAGCTACTGCAGATA-CAGCCGTT-3′) (left panel)). Both splice variants were cloned in the pcDNA3 expression vector (Invitrogen) and caspase-14A and –B were overexpressed in HEK293T cells. Protein extracts were prepared and the presence of caspase-14A and –B in normal human skin was analysed by means of western blotting (right panel)

epidermis, the epithelium of the choroid plexus, the retinal pigment epithelium and Hassall's bodies. Remarkably, the expression of caspase-14 might be confined to epithelia of ectodermal origin. Moreover, the epidermis, the choroid plexus and RPE are epithelia involved in barrier maintenance. Epidermal differentiation is associated with caspase-14 processing into p20 and p10 fragments, probably leading to activation.[4,6] Whether caspase-14 is activated in the other tissues where it is expressed, is not clear to date. Currently, no synthetic or natural caspase-14 substrates to monitor caspase-14 enzymatic activity are known. Further studies will be required to shed light on the precise function of caspase-14 in these different tissues.

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