Article

Caspase-14 expression in the human placenta

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Abstract

Caspase-14 is involved in epidermal differentiation, and previous studies demonstrated abundant expression in the skin. However, the expression of caspase-14 in the human placenta has not been reported. The aims of this study were to determine whether caspase-14 is expressed in the first trimester and term human placenta, and if it is associated with apoptosis in the placenta. Caspase-14 is expressed in the trophoblast cells, and in lower amounts in the mesenchyme. Western blot analysis demonstrated increased expression in the first trimester compared with term placentae. Immunohistochemistry for caspase-14 showed diffuse expression in the trophoblast layer, and not only in occasional cells that are identified by TUNEL staining. Using an explant model in which apoptosis was inhibited with superoxide dismutase (SOD), no significant differences in caspase-14 protein concentrations were seen with differing levels of apoptosis. Caspase-14 is present in the human placenta, primarily in the trophoblast, but its function is not clear, and appears not to be related purely to apoptosis.

Keywords: *apoptosis, caspase, caspase-14, cell death, human, placenta*

Introduction

The human placenta performs a variety of functions to ensure a protected environment for the development of the fetus inside the mother. It regulates the respiratory and nutrient exchange between the mother and fetus, as well as serving as an important endocrine organ that synthesizes various hormones and proteins. The syncytiotrophoblast, the outermost layer of the placenta, is a multinucleated cell layer that forms the principal interface between the maternal circulation and fetal/placental tissues.

Placental apoptosis is a normal physiological process occurring throughout gestation, and occurs in all placental cell types, increasing significantly from the first to the third trimester (Nelson, 1996; Smith *et al.*, 1997). Apoptosis has a critical role in placental development, including interstitial and endovascular invasion (Chan *et al.*, 1999), maternal immune tolerance (Chan *et al.*, 1999), and placental trophoblast turnover (Smith *et al.*, 1997). One of the major apoptotic pathways is through activation of the caspases. Caspase-14 is the newest member identified in the caspase family (van de Craen *et al.*, 1999). Ahmad *et al.* (1998) suggested that caspase-14 might play a role in mouse death receptor and granzyme B-induced apoptosis; however, no human caspase-14 analogues of this function have yet been identified, though a role as a cytokine activator in humans has been suggested (Mikolajczyk *et al.*, 2004).

Caspase-14 is found abundantly in mammalian skin. In human skin, the caspase-14 protein is localized to the differentiating keratinocytes of the skin, supporting a role of caspase-14 in epidermal barrier formation (Chien *et al.*, 2002). In addition, caspase-14 mRNA is expressed only in the uppermost layer of living epidermal cells, including the granular layer, hair follicles and sebaceous glands, suggesting a wider role than a pro-apoptotic gene (Eckhart *et al.*, 2000).

Caspase-14 is also expressed in the epithelium of the human choroid plexus, retinal pigment and in the thymic Hassall's bodies. In the choroid plexus, caspase-14 protein is expressed in the cells that facilitate the transfer of molecules between the blood and the cerebrospinal fluid (CSF), thus forming part of the blood–CSF barrier (Lippens *et al.*, 2003). Caspase-14 is also

expressed in the skin of the embryonic and adult mouse (Kuechle *et al.*, 2001).

To date, a detailed study of caspase-14 in the human placenta has not been reported. This study examined the expression of caspase-14 in the human placenta, and related the expression to apoptotic cells. Using an in-vitro model of apoptosis in first trimester villi, correlation of expression with apoptosis was examined.

Materials and methods

Tissue collection

This study was approved by the ethics committee of King Edward Memorial Hospital (KEMH). First trimester placentae (8–12 weeks of gestation) were collected from legally approved terminations of pregnancy, while term placentae (38–41 weeks of gestation) were collected from uncomplicated deliveries via Caesarean and vaginal delivery. For the first part of the study, these placental samples were obtained within 30 min of delivery or termination and were immediately snap frozen in liquid nitrogen and stored at –80°C, while a separate portion was fixed overnight in 10% neutral buffered formalin (NBF) for immunocytochemical analysis. In the explant study, fresh samples of placenta were transferred onto ice and delivered to the laboratory within 30 min. Human skin for positive control for caspase-14 was obtained from surgical material.

Tissue sectioning

All human placenta and human skin tissue sections were cut uniformly at 5 μm thickness and mounted on silanated slides. Pre-embedded human skin for microscopic analysis was provided by Dr Diane Keeney (Vanderbilt University, Tennessee, USA). Sections were stained in haematoxylin and eosin using routine histological techniques to illustrate tissue morphology in the specimens.

Primary antibody

The polyclonal caspase-14 antibody was provided by Dr Wim Declercq (University of Ghent, Belgium) and was used for both the immunocytochemistry and Western blot analysis. This antibody has previously been used to detect caspase-14 expression in other tissues (Lippens *et al.*, 2003).

Immunohistochemistry

Immunocytochemistry was conducted on normal placental tissue from first trimester (8–12 weeks of gestation, $n = 5$) and from term placentae (38–41 weeks of gestation, $n = 4$). Negative controls were performed on the human placenta and skin, in the absence of the caspase-14 primary antibody. All reagents were diluted in Tris-buffered saline solution (TBS; 0.1 mol/l Tris and 0.15 mol/l NaCl) made up with 0.025% of Tween-20 (TBST-T) and 10% goat serum (Hunter Antisera, Jesmond, NSW, Australia). Slides were heated to 60°C for 15 min and were de-waxed in two washes of absolute toluene for 5 min each, followed by rehydration from 100% ethanol to double distilled water (DDW). After de-paraffinization, the tissue sections were equilibrated in TBS for 5 min. The slides were then rinsed in DDW and subjected to antigen retrieval in a sodium citrate buffer (10 mmol/l; pH 6.0) at 90°C for 20 min. Afterwards, the slides were cooled for 20 min at room temperature and rinsed three times in TBS, for 5 min each. Excess liquid was removed and non-specific binding of the antibodies was blocked by incubating the sections in 20% goat serum (Hunter Antisera) made in TBS-T for 1 h at room temperature. This was followed by three washes of TBS for 5 min each.

After blocking, the caspase-14 antibody was applied overnight at 4°C at a 1:100 dilution, followed by three washes in TBS for 5 min each. Excess liquid was removed and the secondary antibody, biotinylated goat anti-rabbit, was applied at a 1:200 dilution and incubated for 1 h at room temperature. After incubation, excess secondary antibody was rinsed off in three washes of TBS for 5 min each. In the third step, streptavidin labelled with Alexa Fluor 546 dye (BD Biosciences, North Ryde, NSW, Australia) and DAPI were used together at a 1:100 dilution. The sections were incubated in these tertiary reagents for 1 h at room temperature, followed by another set of three TBS washes for 5 min each. To preserve the longevity of the fluorescent dyes, DAKO fluorescent mounting medium (Dako Cytomation, Botany, Australia) was applied to the sections. The slides were cover-slipped, sealed with nail polish and stored in aluminium foil at 4°C until viewing with fluorescence microscopy.

TUNEL

For the staining of apoptotic cell nuclei, the ApopTag® Plus Peroxidase In-Situ Apoptosis Detection Kit (Intergen Company, New York, NY, USA) was used, as previously described (Berg *et al.*, 2002; Lareu *et al.*, 2003). The procedure was according to the protocol for formalin fixed, paraffin embedded tissue, using a 10 min incubation at room temperature for proteinase K digestion (20 μg/ml). Cellular nuclei were counter stained with methyl green. Sections from five first trimester and four term placentae were examined. A negative control was performed by omitting the terminal transferase enzyme from the labelling mixture. A 4-day post-weaning rat mammary gland was used as the positive control included in the ApopTag Plus Kit. Mature nulliparous Wistar rats were obtained from the Animal Resource Centre (Murdoch, Australia).

Microscopy and imaging

Haematoxylin and eosin and TUNEL slides were viewed with a Leica DMRBE microscope. Images were captured using a Nikon DXM1200F digital camera with ACT-1 v2 (Nikon, Garden City, NY, USA) software. Immunostaining was viewed with a Leica DMRBE microscope and images were captured with a black and white digital camera (CCD Cool SNAP ES camera; Photometrics, Huntington Beach, CA, USA). These images were then pseudocoloured using the V++ v4.0 software.

Western blot analysis

Western blot analysis was performed on five first trimester and four term placental tissues. Tissues were homogenized in sodium phosphate buffer (10 mmol/l, pH 7) containing sucrose (0.25 mol/l), EDTA (1 mmol/l), PMSF (1 mmol/l) and trypsin inhibitor (100 mg/ml). Protein concentration of homogenates was measured (Bradford, 1976) and 30 μg resolved by 12% SDS-PAGE and