Lymphatic vessel hypoplasia in fetuses with Turner syndrome

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Turner syndrome is associated with subcutaneous accumulation of fluid in the neck region that can be visualized sonographically from 10–14 weeks of gestation as massively increased nuchal translucency thickness. Possible mechanisms for this increased translucency include dilatation of the jugular lymphatic sacs because of developmental delay in the connection with the venous system, or a primary abnormal dilatation or proliferation of the lymphatic channels interfering with a normal flow between the lymphatic and venous systems. The aim of this study was to investigate the distribution of lymphatic vessels in nuchal skin tissue from fetuses with Turner syndrome compared with fetuses carrying trisomies 21, 18 and 13 chromosomally normal controls. The distribution of vessels was examined by immunohistochemistry using a monoclonal antibody, PTN63, against 5′ nucleotidase and an anti-laminin antibody. In normal control fetuses (n = 6) and those with trisomies 21 (n = 3), 18 (n = 2) and 13 (n = 2), PTN63-positive and laminin-positive vessels were evenly distributed throughout the dermis and subcutis. In Turner syndrome (n = 3), there was a chain of large vessels that stained with both PTN63 and laminin at the border between dermis and subcutis, but there was scarcity of vessels in the upper dermis and the subcutis. Using PTN63 alone, there were no positive vessels in the upper dermis. We conclude that in Turner syndrome lymphatic vessels in the upper dermis are hypoplastic.

Key words: lymphatic vessels/nuchal translucency/5′ nucleotidase/trisomies 21, 18 and 13/Turner syndrome

Introduction

Turner syndrome is associated with subcutaneous accumulation of fluid in the neck region that can be visualized sonographically from 10–14 weeks of gestation as massively increased nuchal translucency thickness (Nicolaides et al., 1994; Snijders et al., 1996). Possible mechanisms for this increased translucency include dilatation of the jugular lymphatic sacs because of developmental delay in the connection with the venous system, or a primary abnormal dilatation or proliferation of the lymphatic channels interfering with a normal flow between the lymphatic and venous systems (Van der Putte, 1977). In normal embryos the main lymphatics develop from the venous walls, but they subsequently lose their connections with the veins to form a separate lymphatic system, except for the jugulo-axillary sacs, which drain the lymph to the venous system (Van der Putte and Van Limborogh, 1980).

In previous studies investigating extracellular matrix molecules in the skin of trisomic fetuses with increased nuchal translucency, we found alterations in a number of structural proteins, i.e. collagen type VI, laminin and collagen type IV (Brand-Saberi et al., 1994a, b; von Kaisenberg et al., 1998a, b). It seems that the mechanism leading to the increase of fluid in the skin in Turner syndrome is completely different from fetuses with trisomies.

A microscopic study examining the lymphatic vessels in the skin of spontaneously aborted fetuses with cervical cystic hygromas reported that in non-Turner fetuses, there were numerous dilated lymphatic vessels, whereas in Turner syndrome, there were very few such vessels. In the skin of normal fetuses with no cystic hygromas, lymphatic vessels were evenly distributed (Chitayat et al., 1989).

The aim of this study was to investigate the distribution of lymphatic vessels in nuchal skin tissue from fetuses with Turner syndrome compared to fetuses with trisomies 21, 18 and 13, that also had increased nuchal translucency and chromosomally normal controls. The distribution of vessels was examined by immunohistochemistry using a monoclonal antibody, PTN63, against 5′ nucleotidase and an anti-laminin antibody. The enzyme 5′ nucleotidase has its highest activity in lymphatic vessels, but it is also present in high endothelial venules of lymphoid tissues (Turner et al., 1987). Laminin is a major component of basement membranes and therefore highlights large lymphatics and both large and small blood vessels. Smaller lymphatics and lymph capillaries are devoid of basement membranes and are therefore not stained using anti-laminin.

Materials and methods

Nuchal skin was obtained from three fetuses with Turner syndrome, three with trisomy 21, two with trisomy 18, two with trisomy 13 and from six normal controls following termination of pregnancy at 11–19 weeks of gestation. The chromosomal abnormalities were diagnosed by chorionic villous sampling, which was carried out after the sonographic detection of increased nuchal translucency thickness at 10–14 weeks. The terminations in the normal controls were carried out at the request of the mothers for psychosocial indications. The study was approved by the hospital ethical committee and tissue
Figure 1. Double staining with monoclonal PTN63 antibody (orange) and polyclonal antibody against laminin (green) of nuchal skin from fetuses with trisomy 21 (a), trisomy 18 (b), trisomy 13 (c) and normal control (d). Big vessels are double-labelled (arrows), while smaller branches are only laminin-positive (arrowheads). Scale bar = 50 µm.

Figure 2. Nuchal skin of fetuses with Turner syndrome. On the left (a) is the picture from single staining with monoclonal PTN63 antibody (red) showing the absence of lymphatic vessels in the upper dermis and a chain of dilated vessels in the junction between the dermis and subcutis. On the right (b) is double staining with both PTN63 (brown) and laminin (green) antibodies demonstrating yellow-orange fluorescence (because of double staining) in the chain of dilated vessels. Vessels in the upper dermis are only laminin-positive whereas subcutaneous fibroblasts are both PTN63- and laminin-positive. Scale bar = 50 µm.
Lymphatic hypoplasia in Turner syndrome fetuses

Figure 3. Parallel sections of nuchal skin of a fetus with Turner syndrome at 11 weeks. Single staining with antibody against laminin (a) and PTN63 antibody (b). There is a chain of dilated vessels at the junction between dermis and subcutis (arrows). Most vessels are both laminin- and PTN63-positive, but there are more laminin-positive areas. Additionally, macrophage-like cells (mc) in the subcutis are PTN63-positive, whereas small vessels in the upper dermis are only laminin-positive (arrowheads). The epidermal basement membrane is also laminin-positive. Scale bar = 50 µm.

Results

In normal control fetuses and those with trisomies 21, 18 and 13, vessels were evenly distributed in the skin. Large vessels in the dermis and subcutis were stained with both PTN63 and laminin antibodies. Laminin was also detected in the epidermal basement membrane (Figure 1a–d). In normal controls, the staining pattern of blood vessels and lymphatics did not change with gestational age from 11–19 weeks.

In nuchal skin from fetuses with Turner syndrome, there was a chain of large diameter vessels that stained with both PTN63 and laminin at the border between dermis and subcutis (Figures 2 and 3). These vessels appeared dilated. In the upper dermis there was a scarcity of vessels. Using PTN63 alone, the upper dermis was devoid of stained vessels (Figures 2a and 3b). Smaller laminin-positive vessels, indicating blood vessels, were present in the upper dermis (Figures 2b and 3a).

Discussion

The findings of this study suggest that in Turner syndrome there is lymphatic hypoplasia in the upper dermis of the nuchal skin, since PTN63-positive structures were absent. In contrast, a few small laminin-positive vessels were found in this area indicating the presence of blood capillaries. The distribution of vessels differs from that in the nuchal skin of trisomy 21, 18 and 13 fetuses, where increased nuchal translucency is a common finding. In these trisomies, the distribution of vessels resembles that of fetuses with normal karyotype.

These findings are compatible with those from the microscopic studies of Chitayat (Chitayat et al., 1989) who reported lymphatic hypoplasia in the skin of Turner fetuses. Further support for lymphatic hypoplasia in Turner syndrome has been provided by studies investigating women with ovarian dysgenesis as a result of a 45XO karyotype; in these cases, lymphangiography revealed hypoplastic lymphatic vessels in the lower limbs, pelvis and retroperitoneal space (Vittay et al., 1980).

The enzyme 5′nucleotidase has been shown to be involved in the phenomena of cell adhesion and migration on laminin and fibronectin (Risse et al., 1989; Stochaj et al., 1989, 1990). It is tempting to speculate that changes in the distribution of these matrix molecules in Turner syndrome may account for

collection was made in accordance with the Polkinghorne guidelines on the research use of fetal tissues (Polkinghorne, 1989). Fetal skin tissues, dissected from the nuchal region, were embedded into OCT-compound (Leica, Bensheim, Germany), snap frozen and stored at –70°C until further study.

Immunohistochemistry

Tissue samples were sectioned serially at 20 µm using a cryocut 2000 (Leica) and collected onto chrome–alum–gelatin coated slides. Sections were air-dried, treated with bovine serum albumin (BSA) in phosphate buffered saline (PBS) to absorb unspecific antibody-binding, rinsed in PBS and incubated with the first antibody (see below) for 60 min at room temperature in a humid chamber.

PTN63 and anti-laminin were used as first antibodies. PTN63, a monoclonal antibody from mouse raised against the PaTu 8902 cell line established from a human pancreatic adenocarcinoma (Flocke et al., 1992), was used at a dilution of 1:4. Anti-laminin polyclonal antibodies, obtained from Sigma (Munich, Germany), were used at a dilution of 1:200 to stain the basement membranes of major vessels and blood capillaries. After rinsing in PBS, the second antibody was applied for 60 min. For the detection of PTN63, a goat-anti-mouse antibody coupled with Cy3 fluorochrome (Dianova, Hamburg, Germany) yielding a red signal was used. Anti-laminin antibodies were detected by a goat anti-rabbit antibody coupled with fluorescein (Dianova) yielding a green signal. Double-labelling was performed with both primary and secondary antibodies applied to the same sections in a sequence. Sections were covered with mowiol (embedding medium; Hoechst, Frankfurt, Germany) and coverslips. They were viewed and micrographs were taken using an epifluorescence microscope (Carl Zeiss, Oberkochen, Germany), and TMY-400 black-and-white films or colour films.
altered distribution of the distribution of the lymphatic vessels or vessels in general. Since 5'-nucleotidase interacts with both laminin and fibronectin and laminin is unchanged between normal and Turner fetuses, it would be interesting to investigate if the distribution of fibronectin is altered in Turner syndrome. If true this may account for decreased migration of lymphangioblasts into the upper dermis resulting in lymphatic vessel hypoplasia.

In summary, we conclude that an aberrant distribution of lymphatic vessels in the upper dermis of the nuchal skin in Turner fetuses may account for the increase in nuchal translucency due to a failure to drain interstitial fluid in this area and due to a congestion of large diameter vessels at the dermis/subcutis junction. These dilated vessels may account for the 'cystic' appearance of nuchal hygroma which is typical of Turner syndrome. Since no animal model for Turner syndrome is available at present, it seems unlikely that it will be possible to perform perfusion studies to investigate further the physiology of lymphatic transport in the near future.

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