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Podoplanin is expressed by a sub-population of human foetal rib and knee joint rudiment chondrocytes

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ABSTRACT

The aim of this study was to determine if podoplanin was expressed by rudiment chondrocytes in human foetal cartilages. Podoplanin was immunolocalised in first trimester human foetal rib and knee joint rudiments to a sub-population of chondrocytes deep in the rib rudiments, tibial and femoral growth plates and cells associated with the cartilage canals of the foetal knee joint rudiments. Lymphatic vessels in the loose stromal tissues surrounding the developing rudiments were also demonstrated on the same histology slides using antipodoplanin (MAb D2-40) and anti-LYVE-1 and differentiated from CD-31 positive blood vessels confirming the discriminative capability of the antibody preparations used. The D2-40 positive rib and knee rudiment chondrocytes were not stained with antibodies to LYVE-1, CD-31 or CD-34 however perlecan was a prominent pericellular proteoglycan around these cells confirming their chondrogenic phenotype. Discernable differences were evident between the surface and deep rudiment chondrocytes in terms of their antigen reactivities detected with MAb D2-40 or antiperlecan antibodies. Binding of the cytoplasmic tail of PDPN to the ERM proteins ezrin, radixin and moeisin may result in changes in cytoskeletal organisation which alter the phenotype of this central population of rudiment cells. This may contribute to morphological changes in the rudiment cartilages which lead to establishment of the primary ossification centres and is consistent with their roles as transient developmental scaffolds during tissue development.

1. Introduction

Podoplanin (PDPN) is a type 1 transmembrane sialomucin glycoprotein which is also known as T1A (Schacht et al., 2003), T1-alpha (Rishi et al., 1995), gp36 (Zimmer et al., 1999) and gp40 (Zimmer et al., 1997). PDPN was independently identified as OTS8 a protein expressed by the mouse osteoblastic cell line MC3T3-E1 upon stimulation with phorbol-12 myristate 13 acetate (Nose et al., 1990) and also known as E11 antigen, a marker for mouse osteoblasts and osteocytes (Wetterwald et al., 1996). The mouse sialoprotein AGGRUS (mouse platelet aggregate inducing factor) (Kato et al., 2003) also shares identity with PDPN as does the neuronal cell protein RANDAM-2 (Kotani et al., 2003), and PA2.26 a small mucin type transmembrane glycoprotein originally identified as a keratinocyte cell surface antigen (Martin-Villar et al., 2005).

PDPN is expressed by rat alveolar type 1 cells (pneumocytes) in the lung (Rishi et al., 1995), PDPN knock-out mice die at birth due to respiratory failure (Schacht et al., 2003). Rat glomerular epithelial cells (podocytes) (Breiteneder-Geleff et al., 1997), mouse keratinocytes (Gandarillas et al., 1997; Zimmer et al., 1995), Madin–Darby canine kidney cells (type 1) (Zimmer et al., 1995), and glutamatergic neuronal cells in mouse cerebrum (Kotani et al., 2003) also express PDPN.

PDPN has a highly glycosylated amino terminal extracellular extension and a cytoplasmic tail which can interact with cytoskeletal components (Breiteneder-Geleff et al., 1997). It is a structural component of podocyte foot processes in glomerular epithelial cells where the charge repulsive effects conferred by it's high anionic charge localisation contributes to glomerular filtrative properties (Breiteneder-Geleff et al., 1997; Matsui et al., 1999). The cytoplasmic tail of PDPN binds to the ERM proteins ezrin, radixin and moeisin inducing cytoskeletal re-organisation, activation of RhoA and promotion of epithelial–mesenchymal cellular transitions (EMTs) (Martin-Villar et al., 2006). PDPN knock-out mice display myocardial defects due to an absence of EMTs in a subset of myocardial cells (Mahtab et al., 2008). EMTs are operative during the loosening of tight gap junctions between epithelial cells leading to their transformation to a more myofibroblastic phenotype.

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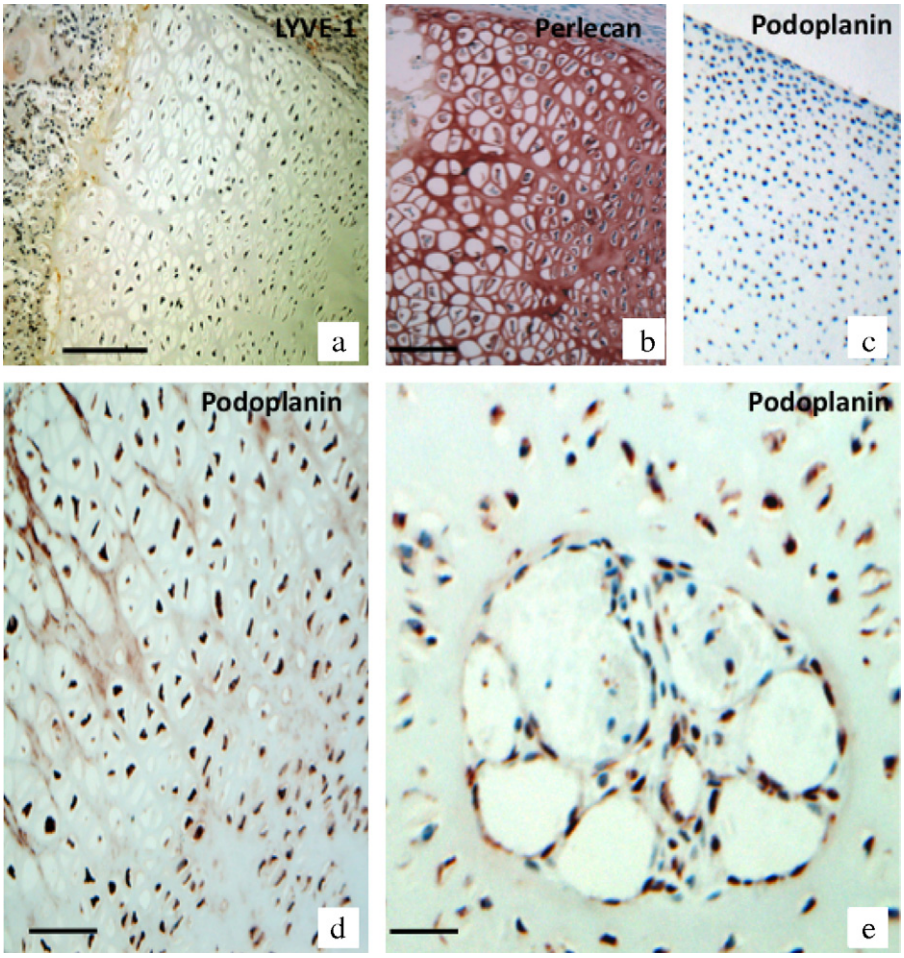

Fig. 1. Growth plate chondrocytes stain negatively with anti-LYVE-1 antibodies (a). Perlecan is a prominently expressed matrix component produced by the growth plate chondrocytes (b). Podoplanin is not expressed by the surface chondrocytes of knee rudiments (c) while the growth plate chondrcoytes (d) and those associated with cartilage canals (e) are stained positively for podoplanin. 12 week old human foetal knee. Scale bars 100 μm. NovaRED chromogen was used for visualisation.

This process is also implicated in the generation of spindle cells in Kaposi's sarcoma and in angiosarcomas (Breiteneder-Geleff et al., 1999). Furthermore, PDPN is up-regulated in a number of human tumors of the colon, rectum, and small intestine (Kato et al., 2003; Ordonez, 2006) and in mesothelioma (Kimura and Kimura, 2005), in osteogenic and chondrogenic bone tumors (Ariizumi et al., 2010) and has roles in lymphangiogenesis (Cueni et al., 2010) and cellular invasion (Cortez et al., 2010) in tumor development.

Table 1
Antibody reactivities of rib, knee and elbow rudiment chondrocytes.

Region/Ab	CD-31	D2-40	LYVE-1	A76
Surface	–	–	–	++
Deep	–	++	–	++
Growth plate	–	++	–	+++
Cartilage canals	–	+	–	++

Table 2
Identification of rib rudiment associated vessels.

Vessel no/Ab	CD-31	D2-40	LYVE-1	A76	Identity
1	++	–	–	+	Venule
2	++	–	–	+	Arteriole
3	++	++	++	–	Lymphatic
4	++	–	–	++	Arteriole
5	++	–	–	+++	Venule
6	++	+	++	–	Lymphatic

2. Materials and methods

Histochoice was an Amresco product (Solon, OH). Anti-perlecan domain-1 (MAb A76) and anti-LYVE-1 were purchased from abcam through Sapphire Bioscience, Sydney, Australia. Anti-CD-31, anti-podoplanin (MAb D2-40), negative control mouse monoclonal IgG1 (clone DAK-GO1) against *Aspergillus niger* glucose oxidase were purchased from DAKO Australia (Botany Bay, Australia).

2.1. Tissues

Rudiments from six 12–14 week old human foetal knee joint and rib specimens were obtained at termination of pregnancy with informed consent and ethical approval of our Institutional Human Research and Care Ethics Review Board.

2.2. Preparation of human foetal tissues for immunohistology

Human foetal knee joints and rib specimens were fixed in Histochoice for 24 h, dehydrated in graded ethanol solutions and embedded in paraplast wax blocks. Vertical or transverse 4 μm microtome sections were cut and attached to SuperFrost Plus glass microscope slides (Menzel-Glaser, Germany), de-paraffinised in xylene (2 changes × 5 min), and re-hydrated through graded ethanol washes (100–70%, v/v) to water.