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Sinusoidal hemangioma and intravascular papillary endothelial hyperplasia: Interrelated processes that share a histogenetic piecemeal angiogenic mechanism

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ABSTRACT

Sinusoidal hemangioma, characterized by interconnecting thin-walled vascular spaces, may present papillae/pseudo-papillae and zones that resemble intravascular papillary endothelial hyperplasia (IPEH). Our objectives are to explore the existence of zones in IPEH with sinusoidal hemangioma characteristics, the mechanism of papillary and septa formation in sinusoidal hemangioma and the comparison of this mechanism with that in IPEH. For these purposes, specimens of 4 cases of each entity were selected and studied by serial histologic sections and by immunochemistry and immunofluorescence procedures. The results showed a) zones with characteristics of sinusoidal hemangioma in IPEH cases, b) presence in both entities of papillae with a cover formed by a monolayer of CD34+ and CD31+ endothelial cells (ECs) and a core formed by either type I collagen and αSMA+ cells (presenting a pericyte/smooth muscle cell aspect) or thrombotic components, and c) a similar piecemeal angiogenic mechanism in papillary formation, including sprouting of intimal ECs toward the vessel wall itself or intravascular thrombi, formation of vascular loops that encircle and separate vessel wall or thrombus components, and parietal or thrombotic papillae development. The major differences between both entities were the number, arrangement and substrate of papillae: myriad, densely grouped, parietal and thrombotic papillae in IPEH, and a linear arrangement of predominant parietal papillae in sinusoidal hemangioma, originating septa (segmentation). In conclusion, sinusoidal hemangioma and IPEH are interrelated processes, which share morphologic findings and a piecemeal angiogenic mechanism, combining sprouting and intussusceptive angiogenesis, and leading to papillary formation and vessel segmentation.

1. Introduction

Sinusoidal hemangioma is a distinctive subset of cavernous hemangioma characterized by interconnecting thin-walled vascular channels (Ban et al., 2010; Blereau, 2009; Calonje and Fletcher, 1991; Ciurea et al., 2011; Enjolras et al., 1998; Halawar et al., 2013; Lee et al., 2001; Nakamura and Miyachi, 1999; Piqué-Duran et al., 2010; Salemis, 2017; Wang et al., 2005). This tumour may present papillae/pseudo-papillae and zones that resemble intravascular papillary endothelial hyperplasia (IPEH) (Calonje and Fletcher, 1991; Halawar et al., 2013). An initial question is whether IPEH also presents zones with characteristics of sinusoidal hemangioma.

In IPEH, several authors have described organized and unorganized thrombi and papilla fronds, which define the process (Albrecht and Kahn, 1990; Amérigo and Berry, 1980; Clearkin and Enzinger, 1976; Hashimoto et al., 1983; Kim et al., 2013; Kreutner et al., 1978; Kuo et al., 1976; Masson, 1923; Salyer and Salyer, 1975; Soares et al., 2008). In a previous work, we reported that the findings in IPEH are the pathologic result of a peculiar neovascularization, by which papillae (folds and pillars) are formed by a piecemeal angiogenic mechanism, combining sprouting and intussusceptive angiogenesis (Díaz-Flores et al., 2016). Intravenous papillary formation by a piecemeal angiogenic mechanism was also demonstrated experimentally by PGE2 and glycerol administration around rat femoral veins (Díaz-Flores et al.,

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2017a). Since sinusoidal hemangioma can show intravascular papillae, a second question is whether papillae/pseudo-papillae in sinusoidal hemangioma may have a similar mechanism of formation to that described in IPEH and in experimental conditions.

In studies of sprouting and intussusceptive angiogenesis, several types of remodelling and pruning have been described in small and large vessels by fold and pillar formation (Djonov et al., 2002; Patan et al., 1996, 2001a,b). One of these remodelling phenomena leads to vessel segmentation. Another question is whether the thin-walled vascular channels in sinusoidal hemangioma are the result of vessel segmentation in which papillary structures may intervene.

Given the above, the objectives of this study were to assess a) the existence of zones in IPEH with characteristics of sinusoidal hemangioma, b) the mechanisms of papillary and septa formation in sinusoidal hemangioma and c) the comparison of this mechanism with that previously highlighted for IPEH and the possible interrelation of these entities. For these purposes, specimens of sinusoidal hemangioma and of IPEH were studied by serial histologic sections, and by immunochemistry and immunofluorescence procedures.

2. Material and methods

2.1. Human tissue samples

The archives of the Department of Anatomical Pathology of the University Hospital of the Canary Islands (La Laguna University) were searched for cases with a diagnosis of sinusoidal hemangioma and IPEH from 2005 to 2017. Specimens of 6 cases of sinusoidal hemangioma and 12 cases of IPEH were obtained and studied by light microscopy. Patients were Caucasian: 4 males and 2 females, aged 25–61 years for sinusoidal hemangioma and 5 males and 7 females, aged 8–71 years for IPEH. For serial histologic sections (20 H&E stained sections in each selected case) and for immunohistochemistry and immunofluorescence procedures, 4 cases of each entity were selected. Ethical approval for this study was obtained from the Ethical Committee of La Laguna University (No. CEIBA2017-0279).

2.2. Light microscopy

Specimens for light microscopy were fixed in a buffered neutral 4% formaldehyde solution, embedded in paraffin and cut into 4 µm-thick sections. Sections were stained with Haematoxylin and Eosin (H&E), Trichrome staining (Roche, Basel, Switzerland. Ref. 6521908001), Reticulin staining (Roche, Ref. 05279399001), Elastin staining (Roche, Ref. 05279216001) and Martius-Scarlett blue (Atom Scientific, Cheshire, UK. Ref. RRSK2-100).

2.3. Immunohistochemistry

Histologic sections, 3 µm-thick, were attached to silanized slides. After pre-treatment for enhancement of labelling, the sections were blocked with 3% hydrogen peroxide and then incubated with primary antibodies (10–40 min). The primary antibodies (Dako, Glostrup, Denmark) used in this study were CD34 monoclonal mouse anti-human, clone QBNEnd 10, (dilution 1:50), catalog No. IR632; CD31 monoclonal mouse anti-human, clone JC70A (dilution 1:50), catalog No. IR610; and α-smooth muscle actin (αSMA) monoclonal mouse anti-human, clone 1A4 (dilution 1:50), catalog No. IR611. The immunoreaction was developed in a solution of diaminobenzidine and the sections were then briefly counterstained with haematoxylin, dehydrated in ethanol series, cleared in xylene and mounted in Eukitt®. Positive and negative controls were used. For the double immunostaining, we used anti-CD34 antibody (diaminobenzidine, DAB, as chromogen) to highlight CD34+ ECs and anti-αSMA (aminoethylcarbazole, AEC, substrate-chromogen) for pericytes/smooth muscle cells.

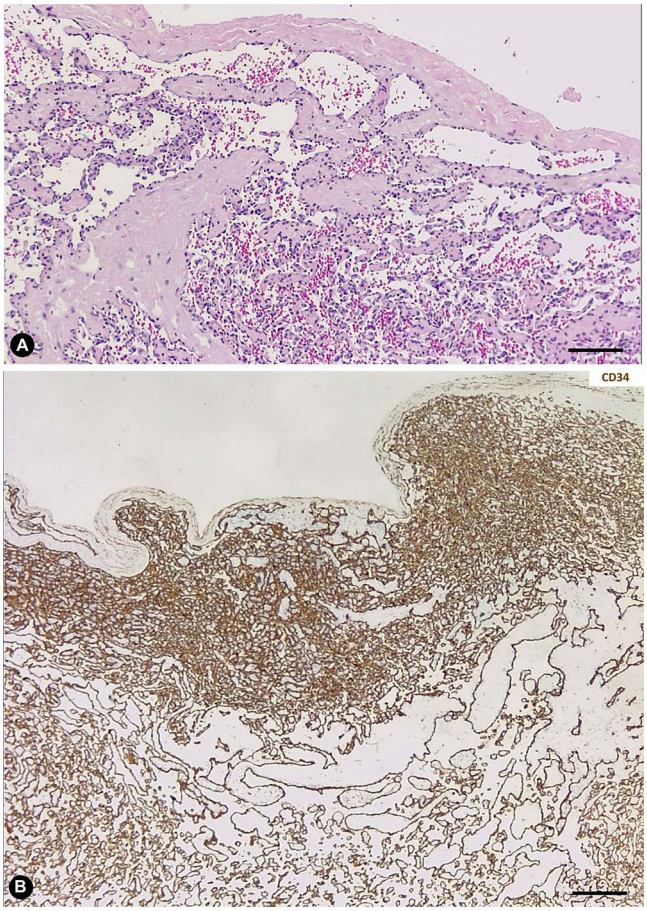

Fig. 1. Zones with sinusoidal hemangioma characteristics in intravascular papillary endothelial hyperplasia (IPEH). A: A hematoxylin-eosin stained section showing a peripheral zone with sinusoidal characteristics (upper region of image) and numerous papillae typical of IPEH (bottom of image). Note intercommunicating vascular spaces separated by vascular space walls (vessel walls of septa) in the zone with sinusoidal characteristics. B: Section immunostained with anti-CD34, in which a zone with sinusoidal hemangioma characteristics is observed within myriad papillae of IPEH. CD34+ endothelial cells line the walls of vascular spaces and papillae. Scale bars: A and B: 90 µm.

2.4. Immunofluorescence in confocal microscopy

For immunofluorescence, tissue sections were obtained as described above. For antigen retrieval, sections were deparaffinized and boiled for 20 min in sodium citrate buffer 10 mM (pH 6), rinsed in Trisbuffered saline (TBS, pH 7.6, 0.05 M), and incubated with the following primary antibodies diluted in TBS overnight in a humid chamber at room temperature: mouse monoclonal anti-CD34, code no. IR63261 (ready to use), rabbit polyclonal anti-collagen type I (1/100 dilution, code AB749P, Millipore). For the double immunofluorescence staining, sections were incubated with a mixture of monoclonal and polyclonal primary antibodies (mouse monoclonal anti-CD34 and rabbit polyclonal anti-collagen type I). The next day, the slides were rinsed in TBS and incubated for 1 h at room temperature in the dark with the secondary biotinylated goat anti-rabbit IgG (H + L) (1:500, Code: 65–6140, Invitrogen, San Diego, CA, USA) and Alexa Fluor 488 goat antimouse IgG (H + L) antibody (1:500, Code: A11001, Invitrogen), followed by incubation with Streptavidin Cy3 conjugate (1:500, Code: SA1010, Invitrogen) for 1 h at room temperature in the dark. Nuclei were detected by DAPI staining (Chemicon International, Temecula, CA, USA). After washing in TBS, sections were exposed to a saturated solution of Sudan black B (Merck, Barcelona, Spain) for 20 min to block autofluorescence. They were rinsed in TBS and were then cover-slipped with DABCO (1%) and glycerol-PBS (1:1). Negative controls were