IV. PEDIATRIC SURGERY

BRIEF REPORT: THE IMPORTANCE OF EP (ENDOTHELIAL CELLS) IN HEMANGIOMAS

A Radulescu[*,1], RE Iacob[1], ES Boia[1], O Adam[1], V David[1], Maria Puiu[1]
[1]University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania
*Saban Research Institute, Childrens Hospital Los Angeles, Department of Developmental Biology-Surgical Research

Abstract

Although hemangioma is the most common infancy tumor, its causes remain unknown. Infantile hemangioma is an endothelial tumor that grows rapidly after birth. The initial proliferation of hemangioma is characterized by the clonal expansion of endothelial cells (ECs) and neovascularization. The following article is a literature review on the importance of EPCs (endothelial proliferating cells) in hemangioma development. Being a subject of interest, ECs were investigated in other diseases and medical conditions but, until now, to our knowledge there are few studies that showed direct evidence of EPCs involvement in human vascular tumors.

Key words: hemangioma, endothelial cells, vascular tumors

Introduction

Most hemangiomas are small lesions, but about 10% grow rapidly and because of their size and/or location (fig. 1) they can be problematic and even life threatening.[7]

The life span of infantile hemangioma is generally divided into a proliferating phase (0-1 year), involuting phase (1-5 years), and an involuted phase (5-10 years).[1,2]

Early proliferating hemangioma is composed of densely packed endothelial cells (ECs). These ECs have been described as "angioblastic" and shown to be more embryonic than neonatal microvascular ECs based on morphology and protein expression patterns.[1]

Its pathology is not well known and two theories are postulated at present: on the one hand an intrinsic defect of the precursor endothelial cells that, through somatic mutation in a gene regulating angiogenesis, develop a phenotype that induces clonal proliferation.[3] On the other hand, it might arise from cells originating in the placenta that embolize in foetal tissue during pregnancy or delivery.[3]

The similarities in antigen expression between haemangioma cells and placenta tissue support this second hypothesis.[3]

According to Hamlat et al.[4] the relatively low oxygen environment, in which the human faeto-placental unit develops, during the first trimester, is necessary to induce vasculo-angiogenesis via embryonic endothelial cells proliferation, since these cells are sensitive to hypoxia and acidosis.[4]

In newborn infants with haemangioma, persistent embryonic primitive endothelial cells, trapped in the intimae underneath the developing vessels and representing "leader" endothelial cells, can stabilise the labile vascular endothelial growth factor mRNA (VEGF mRNA) and produce other angiogenic factors, degrade the underlying basement membrane and invade into the stroma of the neighbouring tissue.[4]

The transition from intra- to extra-uterine life is accompanied by more or less pronounced hypoxia. Consequently, in babies with haemangioma, hypoxia can act as a switch to activate these "leader" endothelial cells and thereby initiate a cascade of reactions.[4]

Yu et al.[1] showed that hemangioma-derived ECs are clonal and exhibit abnormal behavior, suggesting hemangioma arises from clonal expansion of a single EC carrying a somatic mutation. Also they hypothesize that endothelial progenitor cells (EPCs) play a crucial role in the hemangiomagenesis, perhaps as precursors of the clonal ECs.

The aim of their study was to determine whether EPCs are present in hemangioma.

EPCs have been found in bone marrow, blood circulation, fetal liver, and skeletal muscle.[1]

Recent studies suggested that EPCs, hematopoietic stem cells (HSCs), and progenitor cells contribute to embryonic tissue vascularization, postnatal organ regeneration, and tumor neoangiogenesis.[1]

Identification of EPCs relies on specific cell-surface proteins. CD133, also called AC133 antigen and human prominin-1, is a novel human stem/progenitor cell marker. Endothelial markers including CD34, CD31, von Willebrand factor (VWF) and the VEGF KDR are expressed by EPCs, vascular wall-derived mature ECs, and subsets of hematopoietic cells, whereas CD133 is expressed only in progenitor cells.[1]

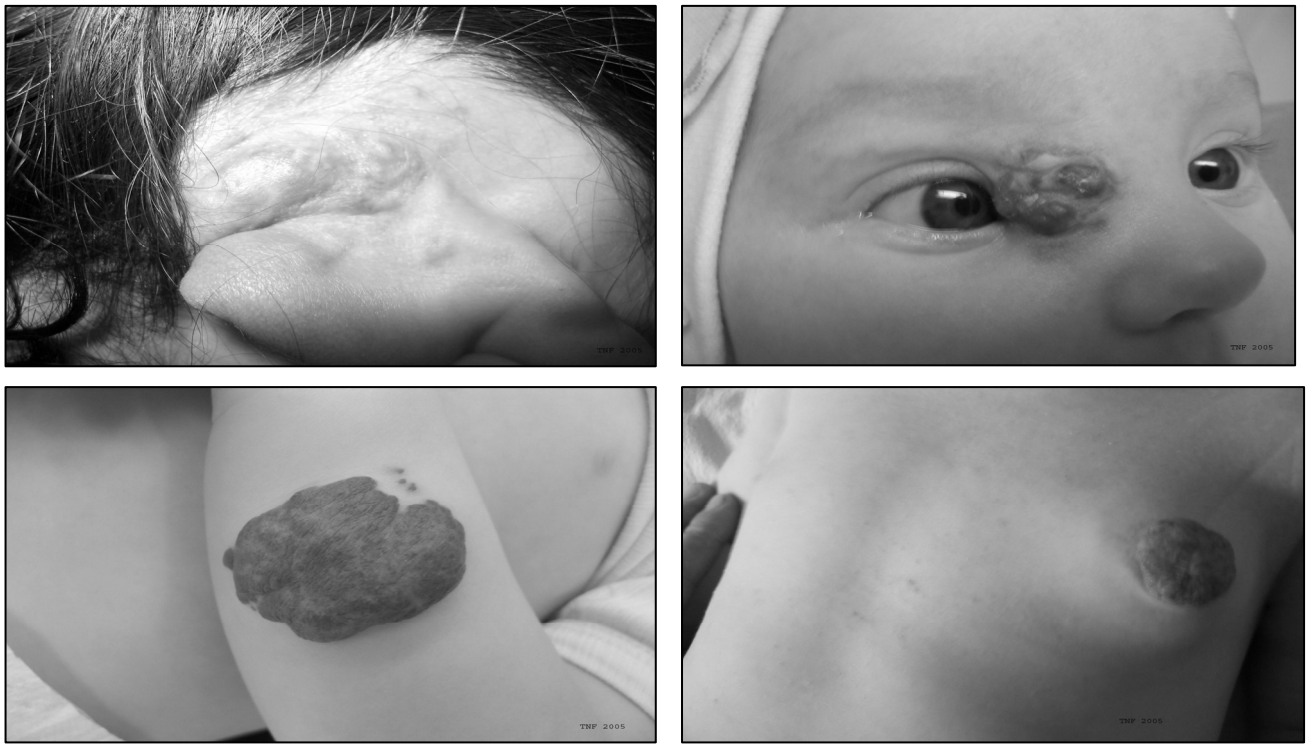

Fig. 1 Hemangiomas located in different parts of the body in 4 patients.

In their study Yu et al.[1] examined CD133 gene expression during hemangioma evolution by Northern blotting and reverse transcription PCR. Using flow cytometry, the investigators showed that proliferating hemangioma contains EPCs that coexpress CD133 and an endothelial marker KDR. These findings suggest that EPCs participate in hemangioma pathogenesis.[1]

In proliferating hemangioma, anti-KDR antibody recognized plump ECs with "immature" morphology, that is large nuclei and scant cytoplasm, lining small nascent vessels but also in the interstitial regions (Fig. 2C). In contrast, flattened KDR^+ ECs were found on the more established vessels in involuting hemangioma (Fig. 2D). The presence of "immature" ECs in proliferating hemangioma is consistent with the CD133 mRNA expression patterns, according to the authors of this study.[1]

Walter et al.[6] suggests that, among EPC in the pathogenesis of the hemangioma a alteration of the VEGF signaling pathway in endothelial and/or pericytic cells could be also to blame.[6]

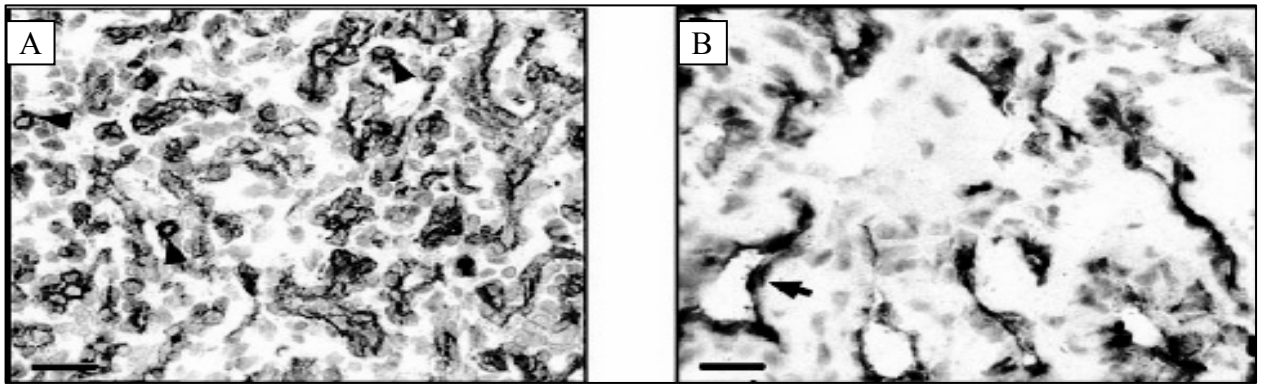

Fig. 2 Endothelial cells evidenced using specific cell-surface proteins: A – intestinal regions, B – involuting hemangioma.

Material and method

The authors of this study investigated the possibility that the tumor is the result of somatic mutation in one or more components of critical vascular growth-regulatory pathways using 15 hemangioma specimens.

Results

Mutations were found in two of the 15 hemangioma specimens: a missense mutation (P1147S) in the kinase domain of the VEGFR2 (FLK1/KDR) gene in one specimen and a missense mutation (P954S) in the kinase insert of the VEGFR3 (FLT4) gene in another specimen. In each case the mutation was detected in tumor tissue but not in adjacent normal tissue.[6]

Identification of EPCs raises the possibility that these cells may give rise to clonal ECs and thereby initiate uncontrolled EC growth. On the other hand we cannot exclude the possibility that EPCs are recruited later from elsewhere during the angiogenesis of proliferating hemangioma.

The question that needs to be answered is if these EPCs, that are involved in hemangioma pathogenesis, originate from bone marrow or a specific tissue.

Boye et al.[7], in an experimental study, showed that endothelial cells from proliferating hemangioma are clonal, and demonstrated that these hemangioma-derived cells differ from normal endothelial cells in their rates of proliferation and migration in vitro.[7] Furthermore, migration of hemangioma endothelial cells is stimulated by the angiogenesis inhibitor endostatin, unlike the inhibition seen with normal endothelial cells.

In an attempt to elucidate the molecular pathogenesis of hemangiomas, the authors tested the intrinsic hypothesis,that the tumors are caused by clonal expansion of vascular ECs. They isolated ECs from proliferating hemangioma in nine infants and the multifocal hemangioendothelioma lesions in one infant.

After that the investigators assayed cell samples from eight of the ten patients for monoclonality and found them all to be clonal, further demonstrating that hemangioma-derived ECs differ from normal ECs in rate of proliferation and migration in vitro, as well as in their response to an angiogenesis inhibitor endostatin.

These results indicate that hemangiomas do indeed constitute clonal expansions of abnormal ECs. The findings are consistent with the possibility that hemangiomas are caused by somatic mutation(s) in a gene(s) that regulates EC proliferation.

Boye et al. [7] concluded that hemangiomas constitute clonal expansions of endothelial cells.

Conclusions

We believe that the data provide support for the hypothesis that hemangiomas are caused by an intrinsic abnormality of ECs. However, it is possible, that all hemangiomas are not due to the same underlying defect. Thus, in some cases, the primary defect could exist external to the proliferating ECs.

It is also possible that even in hemangiomas caused by somatic mutations in ECs, different genes may be involved in different patients. Elucidation of the mutated genes in each case will enhance our understanding of the molecular control of EC proliferation, and the potential for antiangiogenic treatment of hemangiomas.

References

1. Ying Yu, Alan F. Flint, John B. Mulliken, June K. Wu, , Joyce Bischoff "Brief report Endothelial progenitor cells in infantile hemangioma"Blood 15. 02. 2004,vol. 103 nr. 4
2. Mulliken JB. Cutaneous vascular anomalies. SeminVasc Surg. 1993;6:204-218.
3. J. Soto ,"Pathology and pathogenesis of haemangiomas", An. Sist. Sanit. Navar. 2004; 27 (Supl. 1): 27-31.
4. Hamlat A, Adn M, Pasqualini E, Brassier G, Askar B. , " Pathophysiology of capillary haemangioma growth after birth. " , Med Hypotheses. 2005 ; 64 (6):1093-6.
5. Wang H, Zhang Y, Toratani S, Okamoto T. , " Transformation of vascular endothelial cells by a point mutation in the Tie2 gene from human intramuscular haemangioma. ". Oncogene. 2004 Nov 11;23 (53):8700-4
6. Walter JW, North PE, Waner M, Mizeracki A, Blei F, Walker JW, Reinisch JF, Marchuk DA. , " Somatic mutation of vascular endothelial growth factor receptors in juvenile hemangioma. " Genes Chromosomes Cancer. 2002 Mar;33(3):295-303.
7. Eileen Boye, Ying Yu,Gretchen Paranya, John B. Mulliken,Bjorn R. Olsen , Joyce Bischoff , "Clonality and altered behavior of endothelial cells from hemangiomas" J. Clin. Invest. 107:745–752 (2001).

Correspondence to:

Andrei Radulescu,
Iosif Nemoianu Street, No. 2,
Timisoara 300011,
Romania
Phone No. : 0720525832
E-mail : tzutzu77@medical-pa. com