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Clinical Case Report

Metakaryotic cells linked to pediatric pulmonary vein stenosis☆,☆☆

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Pulmonary vein stenosis (PVS) is a serious life-threatening condition with unsatisfactory outcomes with medical and surgical therapeutic remedies [1]. PVS in pediatric patients can occur as a consequence of surgical repair of total anomalous pulmonary venous return (TAPVR) or as a primary condition that can progress spontaneously as can be seen in premature infants [1]. Surgical- and catheter-based approaches to treat PVS unfortunately have a high restenosis [2]. Pathologic specimens of PVS show muscular thickening with increased numbers of smooth muscle cells as well as an abnormal intima layer with spindle cell proliferation by light microscopy which are identified as "myofibroblasts" by routine immunohistochemical analysis [3,4]. Curiously, although proliferation of smooth muscle cells is often reported, there are no reports of smooth muscle cells undergoing mitotic fission. Identification of these pathologic hyperplastic changes in PVS has led to experimental use of cancer chemotherapeutic drugs, but such efforts have not been quite successful [5].

Here we explore the possibility that amitotic "metakaryotic" cells serve as multipotent stem cells creating both pathologic fibroid and smooth muscle cell proliferation. Metakaryotic cells, often identified as "goblet cells," were originally reported to behave as expected for stem cells in human colon development, colonic adenomas, and adenocarcinomas [6]. Metakaryotic cells may be difficult to identify using standard histologic practices. Their distinctive nuclear morphology is usually lost if fixation (Carnoy) is not applied within 15–30 min of surgical extirpation. The cells are generally too large (10×30 μm) to be captured in typical cross sections of 5-μm thickness. The authors of this paper have speculated that metakaryotic cells are likely precursors to myofibroblasts as discussed below. This is the first report of identifying metakaryotic cells in PVS biopsy specimens. Given that a number of common drugs have been shown to specifically kill metakaryotic cells in cell culture, this report offers the possibility of therapy toward this progressive and often fatal disease [7].

1. Methods

Pulmonary vein tissue from five patients with PVS was collected at the time of surgical intervention. The tissue was fixed in Carnoy solution within 30 min of removal from the living patient. Slide preparation with DNA Feulgen staining was carried out in a manner documented previously [8]. Slides were examined under a light microscope for evidence of bell-shaped nuclei, an identifying property of metakaryotic cells. Patient charts were reviewed for clinical history and outcomes. PVS was defined as an elevated gradient (greater than a mean of 2 mmHg by echocardiography) within the confluence of the pulmonary veins as they entered the atrium. Restenosis was defined as a documented decrease from a baseline gradient with subsequent increase at a later date.

2. Results

Patient characteristics are identified in Table 1. Five patients with PVS had biopsy of the stenotic area when undergoing surgical repair of the stenosis. Median age at diagnosis was 4 months. Three of the five had TAPVR with later restenosis in two patients. One had primary PVS, and one had PVS in association with complex congenital heart disease after single ventricle palliation. Two patients died, likely as a result of progressive PVS (patients 1 and 2). One died due to interstitial lung disease (patient 5). The patient with primary PVS (patient 3) had

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Table 1
Patient characteristics

Patient #	Primary diagnosis	Age stenosis discovered	Initial PV surgery (age)	Subsequent PV surgery(ies) (age)	Pathologic finding of MK cells	Progression or persistence of PVS	Disposition
1	Mixed TAPVR; left veins to right middle/lower veins then to right atrium.	2 mo	Direct surgical reimplantation of left veins and sutureless repair of right (7 days)	Direct vein repair ×2, then sutureless repair (2, 3, 4.5 mo)	Yes	Yes	Died. Progressive right heart failure
2	TAPVR to the coronary sinus and single ventricle, transposition	1 day	Sutureless repair (2 days)	PV stenosis resection (3 mo)	Yes	Yes	Died. Pulmonary edema and respiratory failure
3	Primary PVS of left veins and prematurity	4 mo	Sutureless repair (7 mo)	None (n/a)	Yes	Yes	Alive. Restenosis of left veins. Medical tx for PH
4	Double inlet left ventricle. TAPVR to coronary sinus	2.5 years	Surgical unroofing from coronary sinus (2.5 years)	Repeat unroofing from coronary sinus (4 years)	Yes	No	Alive. Heart transplant due to worsening hypoxia, Fontan failure
5	Single ventricle, left pulmonary veins stenosed	1 year	Sutureless repair (1 year)	None (n/a)	Yes	No	Died. Progressive lung disease not due to PVS

Mo, months; tx, treatment; PH, pulmonary hypertension.

complete restenosis with no left-sided pulmonary blood flow but is alive and medically managed for pulmonary hypertension. One patient received a heart transplant due to progressive hypoxia and failure of single ventricle circulation but did not have worsening PVS prior to transplant (patient 4).

Biopsies in all patients showed bell-shaped hollow nuclei cells by light microscopy after Feulgen staining consistent with metakaryotic cells (Fig. 1). Three patients continued to have progressive/persistent PVS after attempts at surgical repair.

3. Discussion

This appears to be the first report that metakaryotic cells have been observed in PVS specimens. The term “metakaryotic” was coined by Drs. Gostjeva and Thilly as these cells appear to fall between prokaryotic and eukaryotic cells in terms of their nuclear organization and division mechanisms [8]. These primitive stem cells were difficult to detect by standard histology techniques. When first reported in the 19th century, they were denominated as “goblet” or “signet ring” cells and ignored as potential stem cells since they were never observed in mitoses. They appear to be an early evolutionary cell type with evidence of their existence found in humans, other animals, and plants [6]. These cells demonstrate symmetrical and asymmetrical amitotic nuclear fission and have partially condensed chromatin in interphase while performing nuclear fission without condensation of most of the chromosomes [8]. They can copy their double-stranded (ds)DNA into an intermediate of dsRNA/DNA prior to and during amitosis and reform dsDNA after segregation of the sister cells [9,10]. Their genomes are organized as closed circles of paired autologous chromatids joined at telomeres but varying among organs and level of differentiation or pathology [7,11]. The new cells created by asymmetric metakaryotic fissions can perform mitotic doublings which create the various differentiated cell types such as those with smooth muscle or fibroid cell properties [9]. In addition to the bell shape of their nuclei, metakaryotic nuclei do not appear to be enclosed by a membrane as are nuclei in eukaryotes; they instead appear to be appended to rather than contained in the cytoplasmic organelle [6]. The metakaryotic cells appear about the fifth week of human gestation, soon form multinuclear tubular syncytia in all organs,

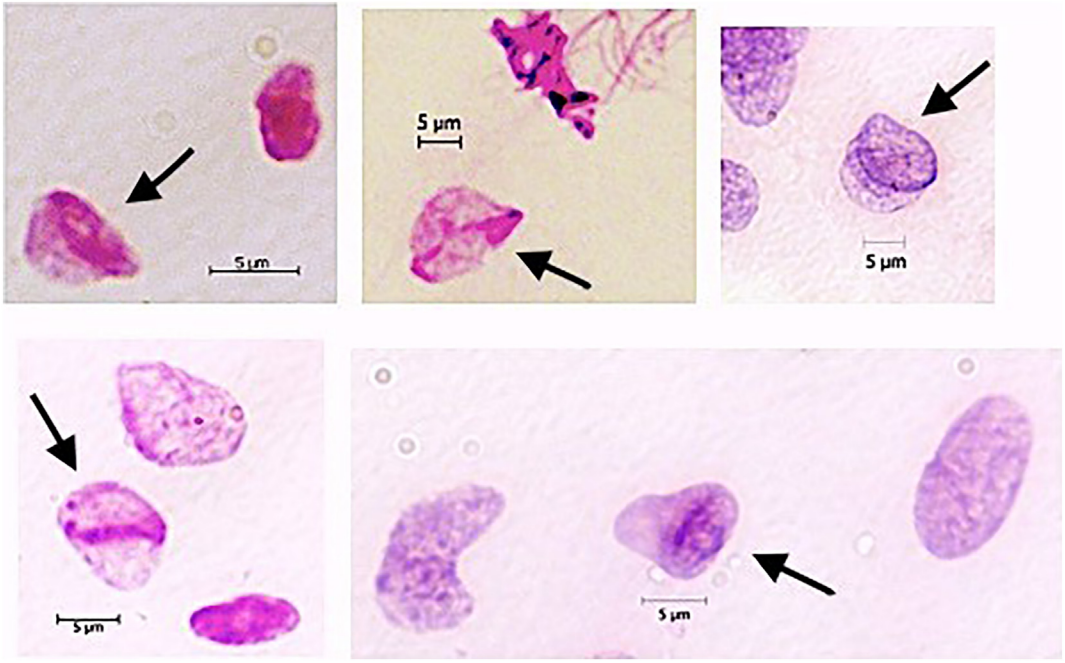

Fig. 1. Feulgen Giemsa staining of cells nuclei in pulmonary vein tissue cells spread by postfixation enzymatic tissue dissociation. Metakaryotic cells with bell-shaped nuclei are present (arrows). Note that the size of the nuclei is larger than 5 µm.

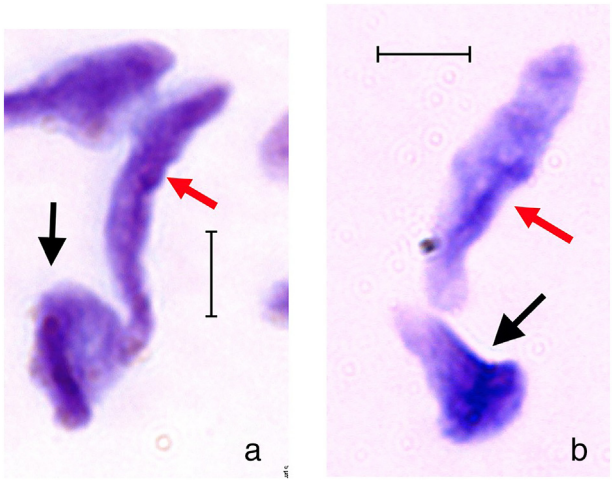

Fig. 2. Asymmetric divisions of metakaryotic stem cells in pulmonary vein stenotic tissue observed in pediatric patients: (a) metakaryotic stem cell with bell-shaped nucleus (black arrow) and emerging spindle-shaped nucleus of a smooth muscle cell (red arrow). Note that the two attached nuclei appear as a pipe-shaped nuclear pair; (b) after nuclear fission, the two nuclei maintain their morphology. Scale bar is 5 μm.

and then undergo metamorphosis into mononuclear forms in all organs save skeletal muscle (where they persist in tubular forms called "myotubes"). Mononuclear metakaryotic cells primarily appear in two main mononuclear configurations: elongated in "goblet" cell form, while others are nearly spherical, a "signet ring" cell form [6].

Prior investigations into the pathology of PVS have shown similarities regardless if it is due to primary (as a result of spontaneous stenosis during the first year of life) or secondary (e.g., after pulmonary vein surgery) etiologies. Pulmonary vein intima was found to have hyperplasia due to fibroblastic (spindle cell) proliferation with a noted loose myxocollagenous matrix [12]. In addition, primary and PVSs associated with TAPVR have histologically found that these intimal spindle cells stain positively for smooth muscle actin and vimentin, giving them the designation of myofibroblasts [3,13]. These findings match prior reporting of the histological characterization of cells derived from asymmetric division of metakaryotic cells [9] (Fig. 2). Even in areas of restenosis, metakaryotic cells have only been found in frequencies of about 2% of all cells, a fraction close to that seen in fetal human organs [5].

The clinical implications of these histological findings as it pertains to patients like ours involve the possibility of a potential new target for therapy in this devastating disease. Primary PVS, as can be seen in premature infants, usually presents with pulmonary hypertension which becomes the main cause of death [14]. Median survival has been estimated at 73% by 1 year and 55% by 2 years, with worse survival the younger a patient is when diagnosed and with increased number of pulmonary veins involved [14]. PVS has been found in 11% of patients with TAPVR preoperatively, with an increased incidence of 18% postrepair, resulting in a decreased 3-year survival in those patients of 59% [15] illustrating disappointing results in surgical-, catheter-, and medical-based approaches to treatment [2,16]. Medical therapy to target myofibroblastic growth has involved the use of chemotherapeutic agents such as methotrexate and vinblastine with significant toxicities and limited evidence of any clinical benefit [5]. One reason for the limited benefits of using a chemotherapeutic approach to treat PVS may be the metakaryotic cells themselves. Gostjeva et al. have found that metakaryotic cells derived from human colonic adenocarcinoma were resistant to these commonly applied chemotherapeutic agents (alkylating, mitocides, and antimetabolites) as well as to radiation therapy [7]. Fortunately, they also found that the metakaryotic cells are susceptible to common medicaments such as certain antibiotics and antihypertensive and diabetes medications, one of which is being investigated clinically to see if it kills human tumor metakaryotic cells in vivo by our colleagues at the Medical College of Wisconsin [7].

4. Conclusions

We report the finding of metakaryotic cells in biopsy specimens in pediatric patients with PVS. We think that they are the likely precursor to myofibroblasts and then fibroid cells that can be seen in PVS. Separately, we think that the metakaryotic cells also create the smooth muscle cells involved in stenosis. We provide evidence that these cells may reasonably be associated with PVS and could be a target of already-discovered "metakaryocides" in the treatment of PVS.

Conflict of interest

There are no conflicts of interest in the creation of this article with its content.

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