Medical Hypotheses 83 (2014) 306–311

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

The epithelial mesenchymal transition process may contribute to the pathogenesis of amniotic band syndrome

M. Romero-Valdovinos [a], N. Bobadilla-Sandoval [b], A. Flisser [c], F. Vadillo-Ortega [d,*]

[a] Departamento de Biología Molecular e Histocompatibilidad, Hospital General "Dr. Manuel Gea González", Secretaría de Salud, Mexico
[b] Unidad de Fisiología Molecular, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México and Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico
[c] Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico
[d] Unidad de Vinculación de la Facultad de Medicina, Universidad Nacional Autónoma de México en el Instituto Nacional de Medicina Genómica, Mexico

ARTICLE INFO

Article history:
Received 20 November 2013
Accepted 4 June 2014

ABSTRACT

The etiology of the amniotic band syndrome is unknown, and has been subject of debate since the time of Hippocrates. The most accepted theories fail to cover all the abnomalities found in affected children. During organogenesis the epithelial–mesenchymal transition process (EMTP) participates in adequate formation of different organs from three embryo layers. Altered activation of EMTP occurs when the epithelial homeostasis is disturbed, the resulting myofibroblasts are able to secrete extracellular matrix proteins and deposit them on the tissues contributing to a fibrotic phenotype. If injury occurs during organogenesis, wound healing could be exaggerated and fibrotic response could be triggered. The molecule that regulates both of these processes (EMTP and fibrosis) is the transforming growth factor β (TGFβ); indeed null animals for TGFβ isoforms show similar defects than those seen in the amniotic band syndrome. Based on documented evidence this review intends to explain how the epithelial mesenchymal transition process may contribute to the pathogenesis of amniotic band syndrome.

Introduction

The amniotic band syndrome (ABS), also known as amniotic rupture sequence, amniotic band sequence or amniotic deformity, adhesion and mutilation complex is an extremely rare condition among live born infants, with prevalence ranging from 1:11,200 [1] to 1:50,579 [2]. The pathogenesis of this disorder remains controversial. The spectrum of congenital defects reported within the ABS entity is extremely variable and includes limb deformations and amputations at various levels, pseudosyndactyly, polydactyl, talips; facial anomalies such as cleft lip (usually bilateral), bizarre midfacial clefts, nasal deformity, bony orbital clefts, hypertelorism, eye-lid colobomas, ptosis, ectropion, lacrimal outflow obstruction, and corneal opacities; cranial deformations including hydrocephalus, microcephaly, asymmetric encephalocele, exencephaly, acrania, acalvaria, and anencephaly; neural tube defects, meningocele, and other internal organ anomalies such as heart defects and genitourinary abnormalities [3–5,6–8]. Familial ABS is associated to hereditary connective tissue abnormalities such as Ehlers-Danlos syndrome type IV and imperfect osteogenesis [9].

One of the most accepted hypotheses on the pathogenic mechanism of ABS involves rupture and repair of the first-trimester amniotic membrane. In this setting, leakage of amniotic fluid results in fetal compression and generation of fibrous bands, which in turn may cause direct mechanical damage to fetal parts (amputation, deformation or constriction) [10,11]. An alternative theory proposes a disruption of the embryonic vascular supply as a possible causative mechanism [12,13]. Considering the pattern of internal anomalies seen in ABS, this syndrome is probably due to the proximity of the affected body regions of the embryo to the damaged amniotic membrane and not to the damage in the circulation of the affected region [14,15]. Fibrous band hypothesis assumes that discrete lesions, caused by adhesion of amniotic fibers, interfere with the development of an early embryo, resulting in the disruption of its basic organization. Alternatively, a mutation in the human equivalent of the mouse disorganization gene [16] may be the underlying cause of severe ABS. Although these hypotheses are accepted, they fail to explain all internal defects found in the fetuses. Moreover, in many cases the presence of malformations not easily account for the disruption produced by an amniotic band, since most have included either intracranial malformations or, more often, various skin spurs, pedicles, bridges or other appendages, with or without polydactyl [17]. A number of arguments can be raised against the latter proposal, including the early

* Corresponding author. Tel.: +52 5553501900.
E-mail address: felipe.vadillo@gmail.com (F. Vadillo-Ortega).

http://dx.doi.org/10.1016/j.mehy.2014.06.002
0306-9877/

timing that would be required to account for some of the associated anomalies. The occurrence of the complete ABS in the absence of amnion rupture can be explained when the fetus has no connection to the amnion [12,14].

In this paper we propose the epithelial–mesenchymal transition process (EMTP) as the mechanism that could explain ABS pathogenesis. This process has been associated with normal tissue development and organogenesis as well as with tissue remodeling and wound healing; EMTP is a developmental route that drives polarized, immotile epithelial cells to acquire apolar and highly migratory fibroblastoid-like features. Outstanding, EMT has also been associated with pathologies like fibrosis and cancer [18–20].

Hypothesis

The fibrous band is the characteristic manifestation of the amniotic band syndrome and may be described as a type of fibrosis. It differs from the classical parenquimatous fibrosis in that it occurs as an exaggerated scar formation in the amnion, affecting the fetus. Tissue fibrosis is generally considered to arise due to a failure of the normal wound healing response [21,22]. ABS can be considered a variation of fibrosis potentially arising as a complication of the rupture of fetal membranes, a pathological condition of pregnancy. Fetal membranes also known as amniochorion are extra-embryonic tissues formed by two strata: the amnion and the chorion. The thin avascular amnion layer is attached to the thicker and more cellular chorion layer. Amnion is composed of five strata: amniotic epithelium, basement membrane, compact layer, fibroblast layer and the intermediate or spongy layer. Chorion is composed of three strata: reticular layer, the basement membrane and the trophoblasts layer Fig. 1 [23,24]. The layer of epithelial cells of the amnion, called the amniotic epithelium is in direct contact with the fetus and anchored on the other side to the basal membrane. Immediately below the basement membrane is a network made of dense fibrous connective tissue called the compact layer, also named the "skeleton of the amnios" (Fig. 1). This layer is composed of interstitial collagen types I and III integrated with type IV, V and VI collagens, creating a strong network structure that maintains the mechanical integrity and function of the amnios [25]. Beneath the compact layer lies the fibroblast layer, which is the thickest of the amniotic layers and it is the cellular source of all collagenous compounds.

The cause of the rupture of the fetal membranes is unknown, but intrauterine infection during pregnancy may lead to weakening of the fibrous components of the amniochorion and the consequent loss of integrity that may be documented with loss of transvaginal amniotic fluid. When this conditions occurs at the end of gestation, usually is accompanied by initiation of labor and delivery but when happening at early phases of gestation repair of the membranes is possible. After injury, new connective tissue needs to be synthesized. During this process, mesenchymal amniotic fibroblasts become activated, proliferate and migrate into the wound and synthesized elevated levels of matrix proteins, including collagen and fibronectin. Mesenchymal cells activated during tissue repair and wound healing come from the fetal membranes in the fibroblast layer of amnion and are equivalent to mesangial kidney cells and stellate liver cells [26,27]. Fibroblasts found in wounds are a specialized form of cells termed myofibroblasts, because they express elevated levels of α-smooth muscle actin (α-SMA) and consequently display a markedly enhanced ability to shrink extracellular matrix [21]. This property of myofibroblasts is necessary for wound closure, but may generate complications as are seen in ABS. Myofibroblasts are present in abundance within fibrotic lesions and thus contribute to the excessive scarring. To understand how the wound healing process develops into fibrotic disease in ABS, it is essential to appreciate how normal tissue repair is controlled and how this process becomes distorted in fibrotic disease.

Normal wound healing is regulated by a complex set of interactions within a network of profibrotic and antifibrotic cytokines, as well as secreted proteins. The profibrotic proteins: TGFβ and connective tissue growth factor (CTGF), and the antifibrotic proteins: TNF-α and interferon ϒ (IFNϒ). TGFβ has been proposed to be a central mediator of the fibrotic response because induces fibroblasts to synthesize and shrink extracellular cell matrix [28]. CTGF, discovered more than a decade ago as a protein secreted by human endothelial cells [29], is induced by TGFβ and is considered a downstream mediator of the effects of TGFβ on fibroblasts [30,31]. Similarly, the Induction of the matrix variant of the protein fibronectin (ED-A FN) by TGFβ occurs through alternative splicing of its transcript [32]. This induction of ED-A FN is required for TGFβ1.

Once myofibroblasts are activated, themselves start secreting TGFβ and thereby can sustain their own activation by a self-stimulatory mechanism that is the basis for the auto perpetuating process characteristic of fibrosis [33]. Fibronectin, in turn, has chemotactic properties that can further stimulate the recruitment of inflammatory cells into the site of injury and, together with collagen I, facilitate EMTP and cell migration; CTGF enhances the TGFβ driven response. Finally, excess deposition of extracellular matrix isolates the parenchyma from its oxygen supply, thus, leading to tissue hypoxia, parenchymal damage, and further stimulation of fibrogenesis [34–37]. The traditional paradigm of fibrogenesis is based on the activation/proliferation of local stromal cells However, recent studies indicate that the exclusive role of resident stromal cells in the development of fibrosis has to be reconsidered because additional mechanisms aimed at the enlargement of the

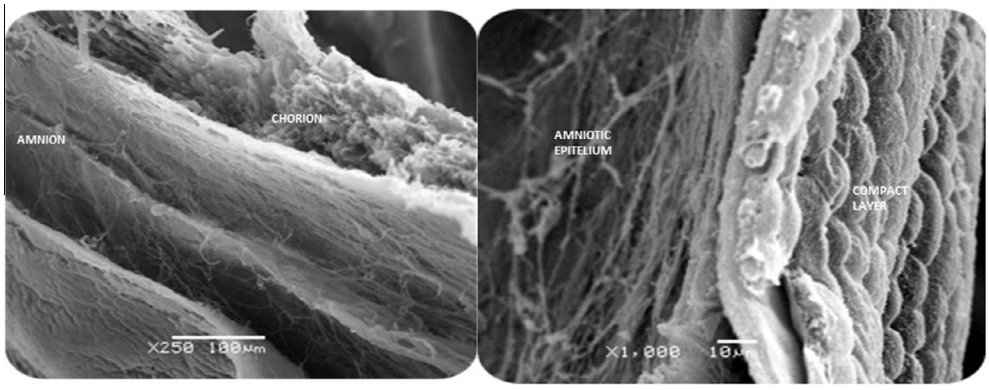

Fig. 1. Scanning electron micrographs of human fetal membranes showing amnion and chorion (left) and amniotic epithelium and compact layer (right) Magnification 250× and 1000×, respectively.