Medical Hypotheses 110 (2018) 64–67

Contents lists available at ScienceDirect

Medical Hypotheses

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

Recently discovered interstitial cells “telocytes” as players in the pathogenesis of uterine leiomyomas

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ARTICLE INFO

Keywords:
Telocytes
Myometrium
Uterine fibroid
Immunohistochemistry

ABSTRACT

Uterine telocytes are interstitial cells characterized by a very long cytoplasmic prolongations, which form a 3D network, functionally integrating a wide variety of different cells. Leiomyomas (uterine fibroids) are benign tumors, which pose a huge threat concerning various health problems in women affected by this condition. The exact cause of leiomyomas development is, however, still largely unknown. Therefore, in an attempt to clarify their etiology, we performed an immunohistochemical characterization of telocytes in leiomyomas as well as in normal myometrium. Tissue samples of intramural leiomyomas from 26 women (age 46.26 ± 11.07) were immunohistochemically stained for the expression of c-kit (CD117) antigen, one of the markers of telocytes. C-kit (CD117) antigen is useful for a routine immunohistochemical identification of uterine telocytes in histological sections of myometrium. In normal, healthy myometrium the c-kit positive telocytes occupy approximately 2.2% of the area of a tissue slide, contrasting with no detectable c-kit positive cells within leiomyomas. As telocytes are thought to be key players in the regulation of tissue homoeostasis, our data suggest that uterine telocyte loss may have important implications in the pathogenesis of leiomyomas. In addition, we supposed to summarize three hypotheses on the association of the cells telocytes loss within the myometrium and formation of leiomyomas. These hypotheses include the loss of telocytes' functions as “sex hormone sensors” and regulators of smooth muscle cells cycle; the role of telocytes as progenitor cells for the development of leiomyomas; and the hypothesis of decreased angiogenesis after telocytes' loss with subsequent hypoxia (as a key factor for leiomyomas development).

Introduction

There have been a huge body of scientific works published recently, concerning a novel population of interstitial cells termed **telocytes.** These cells are characterized by very long cytoplasmic prolongations, which form a 3D network, structurally and functionally integrating immunologically active cells, smooth muscle cells, epithelial cells, nerve fibers and blood vessels. Often these cells are called just ”connecting cells“. This arrangement has been repeatedly found in essentially every organ of the human body including organs of the female reproductive system [1,2]. Many studies describe the role of these cells not only in regulation of physiological functions, but also in plenty of pathological conditions, where the alteration of telocytes is regarded as one of the principal pathological changes. This disruption of normal telocytes physiology is commonly described in terms of their ultrastructure, quantitative changes and modification of their topographical relations with surrounding cells and extracellular components. Focusing on the female reproductive system, dysfunction of telocytes is associated with the pathogenesis of tubal infertility [3,4], endometriosis [5], premature ovarian failure [6], or preeclampsia [7,8]. Several works have been published recently, implying telocytes' dysfunction as one of the key factors also in tumorigenesis [9–11]. Hence, the recent knowledge indicates, that telocytes represent a cardinal cell population with an irreplaceable role in tissue homeostasis and in cell proliferation, differentiation, and survival.

Leiomyomas (uterine fibroids, myomas) are benign monoclonal tumors, which originate from the muscle layer of the uterus (myometrium). Apart from the uncontrolled proliferation of smooth muscle

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https://doi.org/10.1016/j.mehy.2017.11.003
Received 29 September 2017; Accepted 7 November 2017

cells, leiomyomas are also characterized by an increased susceptibility to endocrine stimuli and typical changes in the morphology of extracellular matrix (ECM), with disorganized architecture and alterations in the structure and production of various ECM components [12,13]. Despite their benign nature, these tumors pose a huge threat concerning various health problems in women affected by this condition. The most severe complications associated with uterine fibroids are uterine bleeding, pelvic pain, infertility, miscarriage and a number of other pregnancy-associated complications [14–16]. Moreover, uterine leiomyomas are the most common tumors in women. According to Ahrendt et al. [17], in European population more than 40% of women over 30 years of age have suffered from leiomyomas and more than 50% of all women may develop leiomyomas at some time in their life. For all that, there is a high urgency to acquire a detailed insight into their etiology and pathogenesis.

Intense research of leiomyomas in recent years introduced a number of risk factors and possible causes. The exact cause is, however, still largely unknown. Therefore, in attempt to clarify their etiology, we performed an immunohistochemical characterization of telocytes within leiomyomas as well as in normal myometrium. We searched for an answer about the possible role of these recently described interstitial cells in the pathogenesis of uterine leiomyomas development.

Patients and methods

The specimens of uteruses were taken from 26 women (aged 19–69, mean age 46.26 ± 11.07) who underwent hysterectomy with diagnosis of leiomyomas. The study protocol was approved by the local ethical committee of the General Hospital in Komarno, Slovakia, and informed consent was obtained from every patient. All tissue samples were firstly examined by a pathologist and the diagnosis “intramural leiomyomas” was established. Five control samples of normal myometrium were obtained from the collection of tissue samples available at the Institute of Histology and Embryology, Comenius University in Bratislava, Slovakia. The areas of patients’ tissue samples devoid of leiomyomas were also considered normal for the purpose of our study.

Tissue samples were fixed in formalin for 24 h, embedded in paraffin, and 5 µm thick sections were used for immunohistochemistry. Primary antibodies were used to label and detect the antigen of transmembrane tyrosine kinase receptor c-kit (CD 117) (DAKO, Denmark). Antibodies against c-kit are one of the most routinely used antibodies in the identification of telocytes. The primary antibodies were used according to the manufacturer’s protocols. For visualization, we used EnVision™ FLEX Detection system (DAKO, Denmark) with diaminobenzidine as a chromogen. For better orientation within the slide, cell nuclei were stained with Mayer’s hematoxylin in dark blue. For visualization of histological sections by light microscopy, the LEICA DM2500 microscope was used and images were captured using the LEICA DFC290HD digital camera. The density of area with the positivity for c-kit was calculated via a public domain open source software ImageJ.

Results

Among unstained smooth muscle cells of normal myometrium, telocytes’ cytoplasm showed strong c-kit (CD117) positivity, recognized by brown stain by using diaminobenzidine. The cell bodies of telocytes were spindle-shaped or star-shaped with visible beginnings (proximal portions) of their cytoplasmic projections (Fig. 1). Telocytes were present in all sublayers of myometrium and the average percentage of the positive area within the normal myometrium was 2.19 ± 0.51%. Surprisingly, inside leiomyomas we found **no c-kit positive cells** (Fig. 2). This negativity is not a methodical error. In tissue sections where we could saw both the normal myometrium and leiomyoma in one slide, c-kit positive cells were visible only in those parts of the slide, where the normal myometrium was localized (Fig. 3).

Discussion

Our study showed, that within the normal myometrium the c-kit positive telocytes occupy approximately 2% of the area of a tissue slide. The exact role of uterine telocytes is not clear, but most of the authors suppose their role in intercellular signaling, mechanical and chemical sensation (“hormone sensors”), bioelectrical signaling, modulation of myometrial contractility, or guidance of immune cells [18]. Based on our results, c-kit positive cells are not present within the uterine leiomyomas, only in normal myometrium surrounding the leiomyomas. According to our knowledge, this is the first report about the absence of c-kit positive cells, probably uterine telocytes, inside the uterine leiomyomas. Until now, only two articles have dealt with the association between telocytes and uterine leiomyomas. The first one is a short review article without own results and a valuable conclusion [19]. The second one is from an Egyptian research group, whose conclusions are completely different from ours. Results of Othman et al. [20] show significantly higher count of c-kit positive telocytes within the leiomyomas in comparison to normal myometrium. However, based on the figure captions of the aforementioned cited work, we beg to differ with the results of the authors’ research. Photomicrographs of Othman et al. [20] are somewhat blurred, making it impossible to verify the results – typical feature of telocytes is a c-kit positive cytoplasm, however, their study showed mainly c-kit positive cell nuclei, which is not characteristic of telocytes.

The exact identification of spindle-shaped uterine telocytes in classically stained histological sections, which may be morphologically similar to connective tissue fibroblasts and smooth muscle cells, is impossible. Currently, with the aid of the methods of immunohistochemistry, the identification of telocytes is not so difficult. Various antigens are typical for telocytes, but at present, none of them are considered to be telocyte-specific only [21]. One of these antigens is the c-kit (CD117), which is considered to be the most specific marker of uterine telocytes [22]. However, within the uterus, c-kit is expressed also in mast cells [22], mesenchymal stem cells of endometrium [23] and endometrial cancer stem cells [24]. Nevertheless, development of antibodies against c-kit has allowed for routine identification of uterine telocytes in histological sections.

The proto-oncogene c-kit, encoding a protein transmembrane protein kinase receptor, plays an important role in the signal transduction pathway that regulates cellular growth and repair. The c-kit is highly expressed in number of different neoplasms, e.g. leiomyosarcomas of the uterus [25,26] as well as other uterine sarcomas [27]. It seems, that c-kit may have a significant role in the oncogenesis of mesenchymal tumors of the uterus. In our study we demonstrated, that uterine leiomyomas are absolutely c-kit negative, so they do not contain c-kit positive telocytes. This is surprising because within leiomyomas of the digestive system, some researchers detected c-kit positive spindle-shaped interstitial Cajal cells (note, that telocytes were firstly reported as interstitial Cajal-like cells) [28,29].

Based on the previously described functions of uterine telocytes, we propose three hypotheses of how can be the loss of telocytes within the myometrium involved in the pathogenesis of uterine leiomyomas (Table 1). It is well known, that leiomyoma tissue is more sensitive to estradiol or has more estrogen receptors in comparison to normal myometrium. Thus, leiomyomas can be considered, in general as estrogen-dependent tumors [30,31]. Therefore, increased levels of estrogens have the most significant impact on the development of uterine leiomyomas. On one hand, uterine telocytes act as the sensors of sex hormone levels, as there are progesterone and estrogen receptors present on their surface [32]. On the other hand, telocytes play a role in the regulation of proliferation and apoptosis of smooth muscle cells of the myometrium (the number of telocytes decreases during pregnancy, but it rises again after delivery, as the uterus undergoes involution). [33]. Therefore, the loss of telocytes can disrupt hormonally regulated processes of cellular proliferation and apoptosis within the