ORIGINAL ARTICLE: REPRODUCTIVE SCIENCE

Altered expression of the tachykinins substance P/neurokinin A/hemokinin-1 and their preferred neurokinin 1/neurokinin 2 receptors in uterine leiomyomata

Ayoze González-Santana, M.Sc.,[a] Sara Marrero-Hernández, M.Sc.,[a] Idaira Dorta, Ph.D.,[b] Mariano Hernández, Ph.D.,[b,c] Francisco María Pinto, Ph.D.,[d] Delia Báez, M.D., Ph.D.,[e] Aixa R. Bello, Ph.D.,[b,c] Luz Candenas, Ph.D.,[d] and Teresa A. Almeida, Ph.D.[b,c]

[a] Unidad de Farmacología, Facultad de Ciencias de la Salud, Universidad de La Laguna, Campus de Ofra s/n, Tenerife, Spain; [b] Departamento de Bioquímica, Microbiología, Biología Celular y Genética, Universidad de La Laguna, Spain; [c] Instituto de Enfermedades Tropicales y Salud Pública de Canarias, Universidad de la Laguna, Tenerife, Spain; [d] Instituto de Investigaciones Químicas (IIQ), Consejo Superior de Investigaciones Científicas, Universidad de Sevilla, Sevilla, Spain; and [e] Departamento de Obstetricia y Ginecología, Facultad de Ciencias de La Salud, Universidad de La Laguna, Campus de Ofra s/n, La Laguna, Tenerife, Spain

Objective: To study the expression levels of tachykinins and tachykinin receptors in uterine leiomyomas and matched myometrium.
Design: Laboratory study.
Setting: University research laboratories and academic hospital.
Patient(s): Women undergoing hysterectomy for symptomatic leiomyomas.
Intervention(s): Quantitative polymerase chain reaction, immunohistochemistry and Western blot.
Main Outcome Measure(s): Expression and tissue immunostaining of substance P, neurokinin A, hemokinin-1, neurokinin 1 receptor full-length (NK1R-Fl) and truncated (NK1R-Tr) isoforms, and neurokinin 2 receptor (NK2R) in paired samples of leiomyoma and adjacent normal myometrium.
Result(s): *TAC1* messenger RNA (mRNA) was significantly up-regulated in leiomyomas, whereas intense immunoreaction for the three peptides was particularly abundant in connective tissue cells. Differential regulation of *TACR1* mRNA was observed, and at the protein level there was a significant increased expression of NK1R short isoform (NK1R-Tr). *TACR2* mRNA was significantly up-regulated in leiomyomas, although levels of NK2R protein were similar in normal and tumor cells.
Conclusion(s): These and our previous data demonstrate that the whole tachykinin system is differentially regulated in leiomyomas. The increased expression of NK1R-Tr might stimulate leiomyoma growth in a similar way to that observed in other steroid-dependent tumors. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Leiomyomas, myometrium, NK1 receptor short isoform, tachykinins, tachykinin receptors

Discuss: You can discuss this article with its authors and with other ASRM members at

Mammalian tachykinins (TKs) are a family of biologically active and structurally related peptides derived from three different genes: *TAC1* encodes for substance P (SP) and neurokinin A (NKA), *TAC3* encodes for neurokinin B (NKB), and *TAC4* encodes for hemokinin-1 (HK-1) (1, 2). Tachykinins exert most of their actions by interacting with specific G protein–coupled membrane receptors: neurokinin 1 receptor (NK1R), NK2R, and NK3R encoded by the *TACR1*, *TACR2*, and *TACR3*

Received April 24, 2016; revised June 17, 2016; accepted July 6, 2016.
A.G.-S. has nothing to disclose. S.M.-H. has nothing to disclose. I.D. has nothing to disclose. M.H. has nothing to disclose. F.M.P. has nothing to disclose. D.B. has nothing to disclose. A.R.B. has nothing to disclose. L.C. has nothing to disclose. T.A.A. has nothing to disclose.
Supported in part by research grants from the Agencia Canaria de Innovación y Sociedad de la Información, ACIISI, (PI 2007/001), and from Ministerio de Economía y Competitividad (CTQ2011-25564 and RTC-2014-1431-1), Spain, in both cases with joint financing by FEDER funds from the European Union.
A.G.-S. and S.M.-H. should be considered similar in author order.
Reprint requests: Teresa A. Almeida, Ph.D., Universidad de La Laguna, Departamento de Bioquímica, Microbiología, Biología Celular y Genética, Área de Genética, Campus de Anchieta, Avda. Astrofísico Francisco Sánchez s/n, La Laguna, 38071 Tenerife, Spain (E-mail: tacosalm@ull.edu.es).

Fertility and Sterility® Vol. ■, No. ■, ■ 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.07.007

genes, respectively (3). Substance P and HK-1 bind preferentially to the NK1 receptor, NKA to the NK2 receptor, and NKB to the NK3 receptor. The genes encoding the three TK receptors have a similar structural organization, with five exons expanded by four introns allowing the generation of splice variants (1, 4). Two NK1R receptor isoforms have been reported in humans: a full-length receptor (NK1R-Fl) and a truncated receptor (NK1R-Tr) that lacks 96 amino acid residues at the C terminus (5, 6).

Tachykinins acting through TK receptors contribute to normal homeostasis of respiratory, cardiovascular, immune, endocrine, gastrointestinal, and urinary organ systems. There is also increasing evidence showing that TKs play an important role in the regulation of reproduction (1,7–10). Besides normal physiologic functions, TKs and their receptors have been involved in pathologic conditions, including neoplasia. In cancer cells expressing TK receptors, such as breast, pancreatic, gastric, and colon, TKs promote proliferation and survival (11–14). In addition, activation of NK1R by SP can directly stimulate the process of neovascularization through the induction of endothelial cell proliferation (15). In a large majority of tumors, SP and NK-1 receptors are found in the intra- and peritumoral blood vessels (16), and SP is involved in the growth of capillary vessels in vivo and in the proliferation of cultured endothelial cells in vitro. Recent findings point to NK1R-Tr as a good candidate to mediate malignant transformation in breast and colitis-associated colon cancer (17–19). In addition, a role for NK2R as a potential cell cycle regulator has been proposed (20). Neurokinin A coupled to NK2R activates p53, which in turn is able to bind to the *TACR2* promoter region increasing NK2R expression, leading to cell cycle quiescence of hematopoietic progenitors (20).

Uterine leiomyomas or fibroids are the most common neoplasm of the female genital tract, being present in up to 70%–80% of white and black women by the age of menopause (21). This tumor is composed of various cell types, including smooth muscle cells (SMCs), vascular SMCs (VSMCs), and fibroblasts (FBs), usually surrounded by an enriched extracellular matrix (ECM) (22, 23). Although these tumors are benign, they are responsible for several symptoms, such as heavy or prolonged menstrual bleeding often leading to anemia, pressure symptoms involving increased urinary frequency and pelvic pain, and constipation. In addition, this tumor may interfere with reproduction, because submucosal and intramural leiomyomas that distort the uterine cavity decrease implantation and pregnancy (24). This fact becomes increasingly important becaues the number of infertile women with leiomyomas has increased owing to the delay in child-bearing (25). Moreover, complications during pregnancy and childbirth due to leiomyomas have also been reported (24, 26).

Like most reproductive tract tumors, leiomyomas are steroid hormone dependent, and growth factors, cytokines, chemokines, and ECM components are known factors involved in their pathogenesis (27). Tachykinin and TK receptors are abundantly expressed in the uterus of different mammalian species, including humans, rats, and mice, in which their expression varies with age, during the ovarian cycle, and during pregnancy, and is tightly controlled by ovarian steroids (28–39). In human uterus, TKs acting through NK2R induce myometrial contractions with different orders of potency, NKA > SP ≥ NKB (33). We have previously found that NKB was overexpressed in uterine leiomyoma, it showed a subcellular location different from that found in normal myometrium, and the expression of its high-affinity NK3R receptor was also upregulated, supporting a role for this system in leiomyoma pathophysiology (40). To find out whether the other TKs members (SP, NKA, and HK-1) and their preferred receptors (NK1R and NK2R) could also be differentially regulated in leiomyomas, we have analyzed their expression pattern in leiomyomata at cellular and molecular levels and compared it with the adjacent normal appearing matched myometrium.

MATERIALS AND METHODS

Patients

Eighteen female patients aged 36–49 years, admitted to the Hospital Universitario de Canarias and the Hospital Quirón between 2006 and 2012 were enrolled in this study after giving informed consent. Ethical approval was granted by the Committee for Clinical Research Ethics of the Hospital Universitario de Canarias. Samples analyzed included 16 intramural, submucous, or subserous leiomyoma specimens from 16 women, as well as the matched myometrial tissue; two tumors (intramural and submucous) obtained from one woman, and her matched myometrial tissue; and two tumors (intramural and subserous) obtained from another woman, and her matched myometrial tissue. Myometrial samples were taken as far away as possible from leiomyomata. All patients underwent hysterectomy for menorrhagia without any previous treatment. Regarding the menstrual phase, participants enrolled in this study included 7 in the proliferative phase and 11 in the secretory phase. The proliferative and secretory phases were assigned according to the date of the last menstrual period and confirmed by histologic assessment.

TAC1, *TAC4*, *TACR1-Fl*, *TACR1-Tr*, and *TACR2* mRNA Quantification

RNA extraction and reverse transcription. Gene expression was analyzed in paired samples of leiomyomas and adjacent myometrial tissue (20 tumors and 18 myometrium). To avoid degradation, tissue sections were immersed in RNAlater (Sigma Aldrich) immediately after surgery, kept at 4°C overnight, and stored at −80°C until processed.

Total RNA was extracted using Tri-Reagent (Sigma) and the RNeasy Mini Kit (Qiagen). Fifty milligrams of tissue were homogenized in 1 mL of Tri-Reagent using TissueRuptor (Qiagen). After adding 200 μL of chloroform and mixing, samples were centrifuged at 12,000 × g for 15 minutes at 4°C. One volume of ethanol 100% was added to the supernatant, mixed, and then RNA was cleaned and eluted using the RNeasy Mini Kit according to the manufacturer's instructions. Residual genomic DNA was removed by incubating the RNA samples with RNase free DNase I and RNasin according to the manufacturer's instructions (Promega). The effectiveness of