AROMATASE EXPRESSION IN UTERINE LEIOMYOMATA

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The objective is to determine the alternatively used promoters responsible for aromatase expression in uterine leiomyomata, which is the most common cause of premenopausal hysterectomies in the U.S. Endocrinological studies have revealed that ovarian steroid hormones are essential for the progression of leiomyomata. Aberrant expression of aromatase, the key enzyme responsible for estrogen biosynthesis, has been detected in leiomyoma tissue but not in disease-free myometrial tissue, thereby suggesting that leiomyoma cells synthesize estrogen *in situ*, which, in turn, contributes to the growth advantage of leiomyoma over surrounding myometrium. The aromatase (estrogen synthase) gene is expressed in several extragonadal sites and regulated in a tissue-specific fashion. Its regulatory region contains 10 tissue-specific promoters designated as I.1 (placenta-major), I.2 (placenta-minor), I.3 (adipose/breast cancer), I.4 (skin/adipose tissue), I.5 (fetal tissue), I.6 (bone), I.7 (vascular endothelial cell/breast cancer), PII (ovary/breast cancer/ endometriosis), 2.a (placenta-minor), and I.f (brain). These promoters are used in various tissues by alternative splicing. Use of each promoter gives rise to incorporation of the promoter-specific first exon as the 5'-untranslated end of aromatase mRNA species. Thus, 5'-ends of aromatase mRNA species vary from tissue to tissue and may be viewed as the signature of the promoters used in this particular tissue. The aim of this study is to elucidate the promoters responsible for aromatase expression in leiomyoma tissue.

We isolated total RNA from leiomyoma (n=24) and apparently normal myometrium 2 cm proximal to leiomyoma (n=6) samples as well as myometrium from disease-free uteri (n=1) samples from 23 patients undergoing hysterectomy or myomectomy. These patients were African American, Hispanic or white. We amplified the unknown 5'-untranslated ends of aromatase mRNA by rapid amplification of 5'-cDNA ends (5'-RACE). These sequences were cloned, sequenced and mapped to the genome.

We cloned aromatase mRNA in 14 out of 24 (58 %) leiomyoma samples. A total of 21 promoters are cloned in 14 leiomyoma samples, in which 6 leiomyoma samples showed expression of more than one promoter-specific sequence. The distribution of promoter-specific aromatase mRNA species was as follows: nine promoter I.3 (64 %), four promoter I.6 (28.5%), three promoter 2.a (21.4 %), three promoter II (21.4 %), two promoter I.4-specific mRNA (14.3 %). One myometrial sample adjacent to leiomyoma contained promoter I.3-specific aromatase mRNA (17 %). No amplification was observed in the myometrium from disease-free uterus, as expected.

The primary promoter region responsible for aromatase expression in uterine leiomyomata seems to be promoter I.3/II. These proximal promoters lie within 200 bp from each other coordinately regulated by a PGE2-cAMP-mediated signaling pathway in breast cancer, adipose tissue and endometriosis. In contrast to recently published reports on tissues from Japanese patients, we found a very limited use of promoter I.4, which is different than promoter I.3 in terms of regulation, tissue-specificity and sequence. This may be due racial differences. In our preliminary real-time PCR data, we confirmed the 5'-RACE results by quantifying total or promoter-specific mRNA in 4 leiomyoma samples. Verification of these preliminary results by Real-time PCR in a larger population is under way.