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Effect of estrogen and progesterone on the frequency and spectrum of karyotypically abnormal cells in cultured uterine leiomyomas

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Motivation and Aim: Uterine leiomyomas (ULs), also called "fibroids", are the most common tumors of female reproductive tract [1]. In spite of their benign nature, ULs can rapidly grow to a large size and seriously affect women's health. UL symptoms include pelvic pain, excessive bleeding, infertility and recurrent pregnancy loss. ULs are clonal sex steroid hormone-dependent tumors. Up to 50 % of ULs have chromosomal abnormalities, which are considered to occur secondary during UL tumorigenesis [2]. We recently demonstrated that cytogenetically different UL cell clones have an unequal ability to grow *in vivo* and *in vitro* [3, 4]. In the present study, we aimed to check whether combined and isolated estrogen and progesterone treatment has an effect on both clonal spectrum and clonal proportion in ULs with an abnormal karyotype *in vitro*.
Methods and Algorithms: The experimental approach included conventional karyotyping of ULs cultured in the presence of 10^{-8} M estrogen and/or 10^{-6} M progesterone and without hormones (control) with subsequent verification of the detected chromosomal abnormalities by metaphase and interphase FISH.
Results: We detected karyotype abnormalities in 11 out of 15 ULs cultured without hormones (73 %). In four out of 15 ULs (27 %), no chromosomal abnormalities were found in all cultured samples. The revealed abnormalities were represented exclusively by chromosomal rearrangements, among which interstitial deletions in the long arm of chromosome 7 (del(7q)) were prevalent (n = 6; 40 % of ULs). Rearrangements involving chromosomes 1, 2, 3, 4, 5, 6, 8, 10, 12, 13, 16, 17, 19, and 21 were also observed. In all ULs with abnormal karyotype, more than one clone was detected: two clones with abnormal karyotype were detected in one UL while in the remaining ten ULs clone(s) with abnormal karyotype were combined with 46,XX cells. The number of chromosomally different clones in ULs varied from two to five. A total of twenty different chromosomal abnormalities were detected in 11 ULs. In nine of 11 ULs (82 %) with abnormal karyotype, the spectrum of clones detected in hormone-treated samples differed from the controls. The detected changes were unique for each UL, however, the widest variety of chromosomal abnormalities was detected in control samples as well as in samples cultured using combined estrogen and progesterone supplementation.
We selected six ULs with the most common rearrangement – deletion in 7q – and analyzed the frequency of del(7q) in 1000 cells of each cultured sample using interphase FISH with DNA probes to chromosome 7 (7cen, 7q22, 7q31.2). All samples comprised cells both with and without del(7q). Significant changes in the frequency of del(7q) cells after hormone supplementation were detected in all ULs. Both isolated and combined

estrogen and progesterone supplementation had similar effect on the frequency (either up or down shift) of each clone. Among ULs with one del(7q) clone, two demonstrated significantly increased frequency of del(7q) cells (del(7)(q21q32), del(7)(q21q31),t(2;5)(p13;p13)) after hormone supplementation, while in the rest two ULs, del(7q) clones (del(7)(q21q31), del(7)(q21q31),inv(12)(q15q24)) significantly decreased in number ($p < 0.05$, Chi-square with Yates' correction). One UL with two unrelated deletions in 7q – del(7)(q21q31) and del(7)(q21q36) – showed significantly decreased frequency of both clones after hormone supplementation ($p < 0.05$, Chi-square with Yates' correction). In contrast, clone with del(7)(q11.23q31) decreased in number while clone with 46,XX,del(7)(q21q36) became significantly more numerous after hormone supplementation in other UL ($p < 0.05$, Chi-square with Yates' correction). Notably, for six out of eight del(7q) clones, the changes were more pronounced after isolated but not combined hormone supplementation.

Conclusion: Estrogen and progesterone treatment caused changes in the spectrum of chromosomal rearrangements detected by conventional karyotyping in 82 % of ULs. Interstitial deletions in the long arm of chromosome 7 were the most frequent chromosomal abnormalities ($n = 6$; 55 %). Interphase FISH analysis showed that both isolated and combined estrogen and progesterone supplementation had the same effect on the frequencies of each del(7q) clone in cultured ULs. However, the frequencies of different del(7q) clones may shift up or down in cultured ULs. In most ULs, isolated hormone treatment has more pronounced effect on the frequency of del(7q) cells than combined one. An important finding in our study is that ULs consist of a variety of cell populations, which differ not only karyotypically, but also by their response to sex steroids.

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