行政院國家科學委員會補助專題研究計畫成果報告

P21/WAF1 於子宮頸上皮細胞癌化過程中細胞增生、分化、凋亡之角色

計畫類別：個別型計畫
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執行單位：成功大學醫學院婦產部

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Abstract

Uterine leiomyoma is a common pelvic tumor of reproductive age and sometimes might cause significant morbidity and needs surgical intervention. Preoperatively administration of a long-acting GnRH agonist (GnRH-a) on patients with uterine leiomyomas significantly reduced tumor sizes and thus facilitate surgical removal of large tumors. Whether GnRH-a treatment increases apoptosis in leiomyoma cells is conflicting. Our previous studies indicated that GnRH-a treatment might cause DNA damage of the leiomyoma cells and enhanced Fas ligand (FasL) expression and apoptosis in some cancers. In this article, we investigated on 20 patients having received 3 doses of Lupron, a long acting GnRH-a, before myomectomy and 20 controls the expression of Fas/FasL, caspases including caspases-3, -6, -7, -8, -9 and –10 that involving in initiation, amplification and execution of apoptotic signals from cell membrane, mitochondria, or endoplasmic reticulum and one of the apoptosis-inhibitors, Bcl-2. We found that uterine leiomyomas had up-regulated FasL and caspase-3 levels, and in contrast to the action of GnRH-a on ovarian and endometrial cancers, the levels of FasL and caspase-3 decreased after GnRH-a treatment. The regulation GnRH-a on the expression of FasL protein occurred at post-transcriptional level, whereas that of caspase-3 lied at gene transcription level. The levels of caspases-7, -9, and -10 though not found rise in
leiomyomas, decreased after treatment. There were no fluctuations found on Fas receptor and caspases-6 and −8. Furthermore, the Fas and its ligand were predominantly localized in the cytosol rather in the cell membrane. Leiomyomas had upregulated Bcl-2 and did not change after GnRH-a treatment. Our findings favor the observation that apoptosis is scantly detected in uterine leiomyoma and it is not enhanced after GnRH-a treatment. The expression of caspase-3 and FasL ligand in our condition are associated with tumorigenesis but not apoptosis.

**Key words:** Uterine leiomyoma, gonadotropin-releasing hormone agonist (GnRH-a), Fas, Fas ligand, caspase, Bcl-2, apoptosis.
Fig. 1. Western blotting of tissue lysates of both leiomyomas and myometria of the same patients and leiomyomas after LA treatment of other patients. Immunodetection with FasL antibody revealed a 37kD membrane-bound form (m-FasL, with clone 33 anti-sera) and a 26kD soluble form FasL (s-FasL, with G247-4 anti-sera), which showed that leiomyomas expressed more FasL protein than homologus myometria but down-regulated after LA treatment. Fas did not show consistent alterations of expression in various conditions. N: myometrium. T: leiomyoma; G: leiomyoma after LA (a GnRH-a) treatment.
Fig. 2. Western blotting of tissue lysates of both leiomyomas and myometria of the same patients and leiomyomas after LA treatment of other patients. Immunodetection with antibodies recognized caspases-3, -6, -7, -8, -9, and –10 showed that leiomyomas expressed more caspase-3 protein than homologus myometria but down-regulated after LA treatment. No active caspase-3 which was a procaspase-3 cleavage product with 20 kD in molecular weight was detected in all conditions. Leiomyomas did not have consistent elevation levels of other caspases examined, however, LA-treatment could result in depressive expression of caspases-7, -9 and –10, whereas caspases-6 and –8 were left unchanged. N: myometrium. T: leiomyoma; G: leiomyoma after LA (a GnRH-a) treatment.
Fig. 3. Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of transcription of both Fas-L and caspase-3 genes that showed fluctuations before and after treatment. Total RNA of from both leiomyomas and myometria of the same patients and leiomyomas after LA treatment of other patients were isolated, and transcripts of Fas-L and caspase-3 were analyzed by semiquantitative RT-PCR. At 35 PCR cycles, constitutive transcription of FasL gene found in smooth muscle cells but not altered after tumor formation or after LA treatment whereas mRNA of caspase-3 showed the same fluctuation patterns as those of protein. β-Actin served as quantity control. N: myometrium. T:
Fig. 4. Western blotting of the tissue lysates of both leiomyomas and myometria of the same patients and leiomyomas after LA treatment of other patients. Immunodetection with Bcl-2 (upper panel), and β-actin (lower panel) showed that leiomyoma expressed more Bcl-2 protein than homologus myometria but remained unchanged after LA treatment. N: myometrium. T: leiomyoma; G: leiomyoma after LA (a GnRH-a) treatment.