Regulatory mechanism of *Bcl-2* in uterine leiomyomas

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Summary

Bcl-2 has been thought to play a vital role in the growth of uterine leiomyomas. However, it remains to be fully understood how Bcl-2 expression is regulated in uterine leiomyomas. Several factors have been speculated to affect the induction of Bcl-2 in these cells, including progesterone, endoplasmic reticulum stress, and microRNAs. The elucidation of the regulatory mechanism of Bcl-2 will contribute to a better understanding of the molecular biology of uterine leiomyomas.

Key words: Bcl-2; Leiomyoma; Progesterone; Endoplasmic reticulum stress; MicroRNAs.

The *bcl-2* protooncogene was discovered in lymphoma tumors composed of B cells [1]. Bcl-2 is localized to mitochondrial and perinuclear membranes. Bcl-2 acts to prevent the apoptotic cell death in a variety of cells induced by tropic factor deprivation or other stimuli without altering proliferation [2, 3]. In addition to extending the life span of certain cells, Bcl-2 protein can promote cell replication by reducing the requirements for growth factors [4, 5].

Uterine leiomyoma is the most common benign smooth muscle cell tumor of the myometrium. Bcl-2 protein has been thought to play an important role in the growth of uterine leiomyomas, however, it remains to be fully understood how its expression is regulated in uterine leiomyoma cells. Nonetheless, accumulating data provide evidence that Bcl-2 may be regulated by sex steroid hormones and a pro-apoptotic factor in uterine leiomyoma cells. Furthermore, recent studies raise the possibility that microRNA may be involved in the regulation of Bcl-2 in these cells.

Our laboratory demonstrated that Bcl-2 protein was abundantly present in uterine leiomyoma tissue extracts compared with normal myometrial tissue extracts [6]. This suggests that the greater abundance of Bcl-2 protein in uterine leiomyoma cells may be responsible, at least in part, for the growth of leiomyomas by preventing apoptotic cell death. Moreover, the addition of progesterone resulted in a striking increase in Bcl-2 protein content in cultured leiomyoma cells compared with control cultures, whereas the addition of 17ß-estradiol attenuated Bcl-2 protein content in these cells [6]. Thus, it is speculated that the abundant expression of Bcl-2 protein in uterine leiomyoma cells may be one of the molecular basis characteristic of uterine leiomyomas and that progesterone may play a vital role in the enhanced expression of Bcl-2 protein in these cells.

Other collaborators and I investigated the effects of progesterone receptor modulators on Bcl-2 protein content in cultured uterine leiomyoma cells [7, 8]. We found that progesterone receptor modulators inhibited the cell proliferation of cultured leiomyoma cells and induced apoptosis by up-regulating cleaved caspase-3 and down-regulating Bcl-2 protein content. These data reinforce the progesterone-induced stimulatory action on Bcl-2 protein expression. A recent study has demonstrated that progesterone via progesterone receptor interacts with the *bcl-2* promoter to induce its expression in leiomyoma tissue, suggesting the progesterone-dependent enhancement of growth in uterine leiomyoma [9].

We have recently demonstrated that the induction of endoplasmic reticulum (ER) stress modulates Bcl-2 protein content in cultured leiomyoma cells [10]. The ER is the site of synthesis and folding of secreted, membrane-bound, and organelle-targeted proteins [11]. The conditions that interfere with ER function are referred to as ER stress [11], including the perturbations in calcium homeostasis or redox status, elevated secretory protein synthesis, expression of misfolded proteins, and glucose deprivation [12]. The ER triggers the unfolded protein response to cope with accumulated unfolded or misfolded proteins [12]. However, if the ER stress is prolonged, apoptotic cell death ensues [13]. A pro-apoptotic transcription factor, growth-arrest- and DNA-damage-inducible gene 153 (GADD153), is activated during ER stress [13]. We found that progesterone receptor modulator elicited the ER stress and increased GADD153 protein levels in cultured leiomyoma cells [10]. RNA interference of GADD153 augmented Bcl-2 protein content in cultured leiomyoma cells [10]. This provides further insight into the action of GADD153 in the regulation of Bcl-2 protein in cultured leiomyoma cells.

Lastly, it is postulated that microRNAs (miRNAs) may be involved in the pathogenesis of uterine leiomyomas. MiRNAs are a recently discovered group of small RNA molecules involved in the regulation of gene expression and play a crucial role in diverse functions, including cell proliferation, cell differentiation, stress response, apoptosis, immunity, and transcriptional regulation [14, 15]. MiRNAs function as tumor suppressors (tumor suppressor miRNAs)

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and oncogenes (oncogenic RNAs; oncomiRs). OncomiRs are frequently up-regulated in cancers and show proliferative and/or anti-apoptotic activity [15].

Recent studies have demonstrated that miRNAs are differently expressed in uterine leiomyomas compared with normal myometrium [16-19] and that ovarian steroids modulate the expression of miRNAs [18]. Amomg deregulated miRNAs examined in these investigations, it is noteworthy that oncomiR, microRNA-21 (mir-21), is consistently up-regulated in uterine leiomyomas [16-18]. Knockdown of mir-21 was reported to activate caspases and induce apoptosis in cultured glioblastoma cells [20]. Si *et al.* [21] reported that anti-miR-21 inhibited tumor growth and induced apoptosis in breast cancer MCF-7 cells in association with down-regulation of Bcl-2, suggesting the regulation of Bcl-2 by mir-21. However, the biological significance of mir-21 in the growth of uterine leiomyomas remains unknown. Further studies will be necessary to elucidate the function of miRNAs in uterine leiomyomas.

In conclusion, Bcl-2 is strongly suggested to participate in the growth of uterine leiomyomas by preventing apoptosis. Several factors are thought to regulate the expression of Bcl-2 protein, including progesterone, GADD153, and possibly miRNAs. The elucidation of the regulatory mechanism of Bcl-2 could contribute to a better understanding of the molecular biology of uterine leiomyomas.

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