The ultrastructural investigation of mitochondria in B-CLL cells during apoptosis

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ABSTRACT

BACKGROUND: B-chronic lymphocytic leukemia (B-CLL) is an example of human malignancy caused by alterations in the pathways of apoptosis. Mitochondria play a critical role in the regulation of this process. The B-CLL cells dying in apoptosis showed typical morphological characteristics: the reduction of the nuclear volume is accompanied with the reduction of the cytoplasmatic volume, while many of organelles remain intact. The aim of our study was ultrastructural investigation of mitochondrial morphology in apoptotic B-CLL cells.

METHODS: Our study included peripheral blood samples from 32 B-CLL patients. The samples were fixed in 4% glutar-aldehyde buffered in 0.1 cacodylate buffer and postfixed in 1% osmium tetroxide in the same buffer. The specimens were dehydrated in a graded series of alcohol and embedded in EPON 812. The ultra-thin sections were stained with uranyl acetate and lead citrate. Ultrastructural analysis of sections was performed on Philips electron microscope 208S at 80 kV.

RESULTS: The most frequent mitochondrial abnormalities in apoptotic B-CLL cells were a reduction of size with a hyperdensity of their matrix (mitochondrial pyknosis), or markedly swollen mitochondria with peripherally placed, disoriented, and disintegrated cristae. In some apoptotic cells, we also detected close association of mitochondria with loops of rough endoplasmatic reticulum.

CONCLUSION: The results of our study showed the numerous of mitochondria damages in B-CLL cells during apoptotic process. The correlation between ultrastructural damage and functional activity of mitochondria in apoptotic B-CLL cells is still not clear and requires further investigation.

KEY WORDS: Mitochondria; Leukemia, B-Cell, Chronic; Apoptosis; Microscopy, Electron
PATIENTS AND METHODS

Our study includes peripheral blood specimens from 32 patients with B-CLL (11 untreated and 21 treated). Samples were obtained from 24 men and 8 women. The patients were aged from 50 to 75 yr (61 ± 7.21). B-CLL was diagnosed according to standard clinical and laboratory criteria (23 patients had Binet stage B and 9 stage C disease). The patients with untreated B-CLL were treated by high dose-chlorambucil (HD-CLB). HD-CLB consisted of chlorambucil at the fixed dose of 10mg/daily up to complete or partial remission (CR or PR), or grade 3 toxicity according to the WHO criteria, or a maximum 6 months. Response to therapy was evaluated according to the National Cancer Institute proposal. The patients with treated B-CLL received chlorambucil as maintenance therapy. In the group of untreated patients, we analyzed samples of peripheral blood taken prior to the therapy for detection of spontaneous apoptosis. For detection of therapy-induced apoptosis, we analyzed samples of peripheral blood of treated patients and samples of peripheral blood of untreated patients taken on day 3 of the therapy.

The buffy coat cells (mononuclear cells) in the peripheral blood of patients were enriched by centrifugation (1200 rpm, 15 min) (13). The specimens were fixed in 4% glutar-aldehyde buffered in 0.1 cacodylate buffer (pH7.4) and postfixed in 1% osmium tetroxide in the same buffer. The specimens were dehydrated in a graded series of alcohol and embedded in EPON 812. The semi fine and ultra-thin sections were cut on the LKB ultra microtome III. The semifine sections were routinely stained by 1% toluidine blue in borax. The ultra-thin sections stained with uranyl acetate and lead citrate (14). Ultrastructural analysis of sections was performed on Philips electron microscope 208S at 80 kV.

RESULTS AND DISCUSSION

Ultrastructurally, the lymphocytes in CLL exhibited a high nuclear cytoplasmic ratio. The nucleus had finely dispersed chromatin with condensation at the periphery. Nucleoli are identified in several cells. The cytoplasm contained mitochondria, which showed variation in size and shape, ribosomes and very few strands of rough endoplasmic reticulum. The cytoplasmic margin had a few short projections (Figure 1).

In recent years, many experimental studies demonstrated capability of chlorambucil to induce apoptosis. Chlorambucil is able to induce typical features of apoptosis in B-CLL cells (15,16). There are no morphologically clear differences between the cells dying in the spontaneous or therapy induced apoptosis. The noted differences are more likely to be the differences in the morphologic characteristics of the cells that enter the apoptotic process, rather than the characteristics of the programmed cell death.

Apoptosis characteristically affected scattered single B-CLL cells rather than tracts of contiguous cells. The B-CLL cells of analyzed samples dying in the process of apoptosis showed typical morphological characteristics of this process, as described by many authors and our previous investigations. The apoptotic B-CLL cells initially showed decreased volume and margination, highly osmiophilic chromatIN. A total condensation of the nuclear chromatIN and the reduction the nuclear volume occur at the later phases of the apoptotic process. The reduction of the nuclear volume is accompanied with the reduction of the cytoplasmatic volume, while many of organelles remain intact.

Mitochondria are "life-essential” organelles for the production of metabolic energy in the form of ATP (9). In some apoptotic cells, we detected close association of mitochondria with loops of rough endoplasmatic reticulum (MER complex) (Figure 2).
our previous experimental studies demonstrated increased level of expression Bax protein in peripheral blood of patients with B-CLL (18). These facts leave many questions unanswered about their physiological relevance. Is the clustering of mitochondria close to the nucleus crucial for generating high ATP levels in a domain rich in energy-dependent apoptosis events or is it to facilitate the translocation of mitochondrial proteins (e.g., ATP) to the nucleus? (10).

According to standard morphological descriptions, mitochondria were long thought to remain unchanged during apoptosis but swell during necrosis (10). Mitochondrial morphology is important because changes in mitochondrial ultrastructure modulate mitochondrial function (19). Mitochondrial integrity is central to both caspase-dependent and caspase-independent cell death. The release of pro-apoptotic factors from the mitochondrial intermembrane space is a key event in a cell’s commitment to die and is under the tight regulation of the Bcl-2 family (20).

The apoptotic B-CLL cells showed various ultrastructural damages of mitochondria. The most frequent mitochondrial abnormalities in apoptotic B-CLL cells were a reduction of size with a hyperdensity of their matrix (mitochondrial pyknosis) (Figure 3). Mitochondrial pyknosis as specific alternation of mitochondrial morphology correlate with mitochondrial dysfunction.

In some mitochondria, we have also noted marked swelling with peripherally placed, disoriented, and disintegrated cristae (Figure 4). Mitochondrial swelling is always an osmotic process that results from net solute and water diffusion towards the matrix. Swelling of the matrix would be required to make more cytochrome c available for release into the cytosol (8).

**Figure 4. B-CLL in the process of apoptosis (16,000 x) (arrow = mitochondria with peripherally placed, disoriented and disintegrated cristae)**

The correlation between ultrastructural damage and functional activity of mitochondria in apoptotic B-CLL cells is still not clear and requires further investigation. The results of pioneering works of Wang Xiaodong showed that the mitochondrial damage might also passively lead to cell death due to loss of mitochondrial function (11).

**CONCLUSION**

Mitochondria play a key role in the pathways to apoptosis. Mitochondrial morphology is important because changes in mitochondrial ultrastructure modulate mitochondrial function. The results of our study showed the numerous of mitochondria damages in B-CLL cells during apoptotic process. The most frequent mitochondrial abnormalities were mitochondrial pyknosis and mitochondrial swelling. These facts leave many questions unanswered about their physiological relevance.

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**REFERENCES**