Western Blot Determination of Caspase-3 Apoptotic Activity in Complete Hydatidiform Mole and Persistent Trophoblastic Disease

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Abstract

Objective: Complete hydatidiform mole is a gestational trophoblastic disease. It has the potential for malignant transformation. The precise mechanism of pathogenesis remains poorly understood but it has been suggested that defects in apoptosis may play a role. Most complete hydatidiform moles spontaneously regress after suction evacuation. However, about 8-30% of these patients will develop persistent trophoblastic disease and require chemotherapy. Apoptosis, or programmed cell death, is a biological process that plays a fundamental role in embryonic development, cellular differentiation, and the maintenance of tissue homeostasis. Caspases, a family of cysteine proteases plays a central role in execution of apoptosis. The aim of this study was to assess apoptotic activity of caspase-3 in complete hydatidiform mole and to compare this with persistent trophoblastic disease.
Materials and Methods: In this study, we used Western blot determination of caspase-3 activity to detect apoptosis in samples taken from 3 patients with complete hydatidiform mole, 2 patients with persistent trophoblastic disease and in the placentas of 3 healthy pregnant women as controls.
Results: A dramatic increase in the level of caspase-3 activity was found in 3 patients with complete hydatidiform mole and 2 patients with persistent trophoblastic disease as compared to controls. On the other hand, the band levels of active caspase-3 were found to be lower in patients with complete hydatidiform mole than persistent trophoblastic disease group.
Conclusion: Apoptosis is important in the pathogenesis of complete hydatidiform mole and may be considered as a prognostic indicator for predicting the clinical behavior of complete hydatidiform mole.

Keywords: gestational trophoblastic disease, apoptosis, caspase-3, Western blot

Özet

Komplet Mol Hidatidiform ve Persiste Trofoblastik Hastalıkta, Western Blot ile Kaspaz-3 Apoptotik Aktivitesinin Belirlenmesi

Amaç: Gestasyonel trofoblastik bir hastalık olan komplet mol hidatidiformun maligniteye dönüşüm potansiyeli vardır. Kesin patogenetik mekanizması yeterince anlaşılamamış olmakla beraber, apoptozisteki defektlerin rol oynayabileceği öne sürülmektedir. Komplet mol hidatidiform, vakum evakuasyon sonrası çoğunlukla spontan olarak geriler. Ancak, bu hastaların %8-30'unda, kemoterapi gerektiren persiste trofoblastik hastalık gelişir.
Apoptozis veya programlı hücre ölümü, doku homeostazının sağlanması, hücre farklılaşması ve embriyonel gelişimde temel rol oynayan biyolojik bir süreçtir. Kaspaz ailesi, apoptozun yürütülmesinde önemli rolü olan sistin proteazlardır.
Bu çalışmada, komplet mol hidatidiform ve persiste trofoblastik hastalıkta kaspaz-3 aktivitesini karşılaştırmayı amaçladık.
Materyal ve Metot: Çalışmamızda, üç komplet mol hidatidiform olgusu ile iki persiste trofoblastik hastalık olgusundan alınan örneklerde ve kontrol olarak, sağlıklı üç gebenin plasentalarında, Western blot ile kaspaz-3 aktivitesini araştırdık.
Sonuçlar: Kontrol olgularıyla karşılaştırıldığında, komplet mol hidatidiformlu üç olguda ve persiste trofoblastik hastalıklı iki olguda kaspaz-3 aktivite düzeyinin belirgin olarak arttığı belirlendi. Diğer yandan, komplet mol hidatidiform hastalarında aktif kaspaz-3 bant düzeyleri, persiste trofoblastik hastalık grubundan daha düşük bulundu.
Tartışma: Komplet mol hidatidiform patogenezinde apoptozis önemlidir ve komplet mol hidatidiformun klinik davranışının öngörülmesinde prognostik bir gösterge olarak dikkate alınabilir.

Anahtar sözcükler: gestasyonel trofoblastik hastalık, apoptozis, kaspaz-3, Western blot

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Introduction

Complete hydatidiform mole is a gestational trophoblastic disease in which the genome contains solely paternal contribution and which has the potential for malignant transformation. Most complete hydatidiform moles spontaneously regress after suction evacuation. However, about 8-30% of these patients will develop persistent trophoblastic disease and require chemotherapy.

The precise role and mechanism of genomic imprinting remains poorly understood (1). However, increased apoptotic activity has been shown in gestational trophoblastic disease in several studies (2-4).

Apoptosis is triggered by a wide range of extracellular and intracellular signals and is accompanied by a characteristic set of morphological and biochemical phenomena which include cell shrinkage, membrane blebbing, protein cleavage and DNA fragmentation, which culminate in the formation of apoptotic bodies, membrane-bound cell fragments which are rapidly engulfed by surrounding cells. The molecular mechanisms that control apoptosis are extremely tightly regulated. Two of these control mechanisms have been implicated in the pathogenesis of gestational trophoblastic disease: caspase activity, and the activities of the pro- and anti-apoptotic genes *bax* and *bcl*-2.

The caspases are a family of cysteine proteases that play a central role in the execution phase of apoptosis (5). Caspases initiate the cellular breakdown process by degrading specific structural, regulatory and DNA repair proteins (6). Although apoptosis seems to uniformly require the participation of caspases, the particular caspase utilized varies according to the cell type and the apoptotic stimulus (7). Among these proteases, caspase-3 is probably best correlated with apoptosis since it is commonly activated by numerous death signals and cleaves a variety of important cellular proteins (8).

Recent studies of apoptosis in gestational trophoblastic disease have employed the TUNEL technique (*in situ* nick-end labeling) for assessment of apoptotic activity either on tissue sections or by flow cytometry (2,4,6). Chiu et al (6) assessed apoptotic activity in gestational trophoblastic disease by the caspase-related M30 index using an immunohistochemical technique.

The aim of this study was to assess apoptotic activity of active caspase-3 in complete hydatidiform mole and to compare this with persistent trophoblastic disease using Western blotting.

Materials and Methods

Samples were taken from three patients with complete hydatidiform mole, two patients with persistent trophoblastic disease immediately after evacuation and the diagnosis confirmed by histopathology. Placenta samples were taken from three healthy women undergoing first-trimester termination of pregnancy, immediately after the termination. All samples were stored at -80°C until processed.

The samples were homogenized in lysis buffer (Tris-NaCl-EDTA-DDT-PMS) using a glass Dounce homogenizer. The extracted proteins were precipitated by adding an equal volume of 20% TCA and incubating at 4°C for 30 min, followed by centrifugation at 10,000 rpm for 15 min at 4°C to pellet the proteins. The pellets were washed with 300 µL of cold acetone, re-centrifuged, re-suspended in SDS-PAGE loading buffer, heated at 65°C for 5 min and run on SDS-PAGE using the Mini Protean II system (Bio-Rad, USA).

After electrophoresis, the proteins were transferred to PVDF membrane (Bio-Rad, USA) using the Trans-Blot Semi-Dry system (Bio-Rad) at 20 V for 30 min. The membrane was blocked in Roti-Block reagent (Roth, Germany) with gentle shaking at RT for 1 h. After washing three times with TBS-T (20 mM Tris, pH 7.5, 150 mM NaCl), the blots were incubated with anti-caspase-3 antibody (reactive only with the 17 kDa subunit, Chemicon AB3623) at a dilution of 1:200 for 2 h, washed three times with TBS-T (20 mM Tris, pH 7.5, 150 mM NaCl, 0.05% Tween 20), then incubated with a goat anti-rabbit antibody conjugated to alkaline phosphatase at a dilution of 1:20 000 for 1 h.

After the blots were washed three times with TBS-T and once with TBS, the color reaction was performed in the dark with NBT/BCIP alkaline phosphatase substrate (Sigma Fast tablet) with agitation for 10 to 30 minutes until color development. The color reaction was stopped by washing in several changes of distilled water and the blots were then air-dried and stored in plastic sleeves in the dark.

Results

A dramatic increase in the level of caspase-3 activity (active p17 fragment) was found in 3 patients with complete hydatidiform mole and 2 patients with persistent trophoblastic disease as compared to controls. On the other hand, the band levels of active caspase-3 were found to be lower in complete hydatidiform mole patients than persistent trophoblastic disease group (Figure 1).

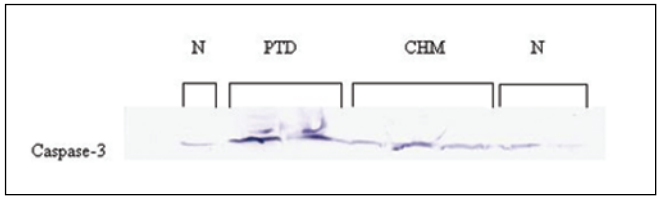

Figure 1. Western blot analysis for the active form of caspase-3 fragment (17kDa), in normal first trimester placenta (N), persistent trophoblastic disease (PTD), and complete hydatidiform mole (CHM), showing a significant increase in the amount of active caspase-3 fragment in complete hydatidiform mole and persistent trophoblastic disease groups compared with controls. In complete hydatidiform mole patients the band levels of active caspase-3 were found to be lower than persistent trophoblastic disease subjects.

Discussion

From a search of the literature, this appears to be the first study to assess apoptotic activity in complete hydatidiform mole by utilizing Western blotting for the detection of active caspase-3. It has been reported that active caspase-3 induces fragmentation of the nuclear DNA and contributes to cell death (5). A previous study (6) demonstrated increased expression of the caspase-related antigen M 30 and showed that the level of apoptotic activity in complete hydatidiform mole can serve as a prognostic indicator for the clinical outcome.

Apoptosis, or programmed cell death, is a biological process that plays a fundamental role in embryonic development, cellular differentiation, and the maintenance of tissue homeostasis. Dysregulation of apoptosis can result in developmental abnormalities and inappropriate cellular proliferation, such as that occurring during cancer. A family of cytoplasmic proteases, the caspases, play an important role in its execution. So far, sixteen distinct caspases have been identified, of which caspase-3 is one of the best characterized. It is synthesized as an inactive proenzyme (32 kDa) that is processed, either by self-proteolysis or cleavage by upstream proteases (such as caspase-9), in cells undergoing apoptosis. The processed form of caspase-3 consists of a large (17 kDa) and a small (12 kDa) subunit, which associate to form the active enzyme (5).

In this study, levels of active caspase-3, as determined by Western blot, were greatly elevated in patients with persistent trophoblastic disease and complete hydatidiform mole compared with normal controls, indicating a possible role for apoptosis in complete hydatidiform mole.

Apoptosis is important in the pathogenesis of complete hydatidiform mole and may be considered as a prognostic indicator for predicting the clinical behavior of complete hydatidiform mole – i.e. progressing to more invasive forms of the spectrum. Further confirmatory studies in larger groups are required to determine if assessing the apoptotic activity can predict the progress of gestational trophoblastic diseases.

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