Detection of Apoptosis Feature in Ultraviolet Light-Exposed *Trichophyton rubrum*

ULTRAVİOLE IŞININA MARUZ KALAN TRICHOPHYTON RUBRUM'UN APOPİTOZ ÖZELLİĞİNİN DEĞERLENDİRİLMESİ

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Abstract

Objective: The aim of this study was to detect the apoptosis feature following the ultraviolet (UV)-light irradiation in *Trichophyton rubrum* and analyze the results in molecular aspect.

Material and Methods: The colonies of *T. rubrum* were irradiated by UV light at a wavelength of 302 nm. For observing the possible apoptosis feature, the high-molecular-weight DNA was isolated from non-irradiated and irradiated colonies of *T. rubrum* respectively and then were run through 1% agarose gel.

Results: In the investigation of isolated DNA molecules, no differences between DNA banding patterns were observed.

Conclusion: By the performed protocol in this project, no phenomenon of apoptosis was detected in the dermatophyte fungus of *T. rubrum*. The negative tropism of *T. rubrum* towards UVB may be the cause of no-apoptosis feature.

Key Words: Apoptosis, *Trichophyton*

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Özet

Amaç: Bu çalışmanın amacı, *Trichophyton rubrum*'un ultraviole (UV) ışınına maruz bırakılmasının ardından apopitoz (hücre ölümü) özelliğini saptamak ve bulguları, moleküler açıdan analiz etmektir.

Gereç ve Yöntemler: *T. rubrum* kolonileri, UV ışını ile 320 nm dalga boyunda ışınlamaya tabi tutulmuştur. Olası apopitoz özelliğini gözlemlemek için, *T. rubrum*'un radyasyon uygulanmış ve uygulanmamış kolonilerinden yüksek molekül ağırlıklı DNA soyutlanmış ve %1 agaroz jelde yürütülmüştür.

Bulgular: Soyutlanmış DNA moleküllerinin incelenmesi, DNA bant şablonları arasında hiçbir fark olmadığını ortaya koymuştur.

Sonuç: Bu projenin protokolü ile dermatofit mantar olan *T. rubrum*'da apopitoz fenomeni gözlenmemiştir. *T. rubrum*'un UVB ışınlarına negatif tropizmi, bu durumdan sorumlu olabilir.

Anahtar Kelimeler: Hücre ölümü, *Trichophyton*

Between 1962 and 1964 two types of cell death were distinguished: Classical necrosis, and a process involving conversion of scattered cells into small round masses of cytoplasm that often contained specks of condensed nuclear chromatin. During 1971-1972, John F.R. Kerr collaborated with Andrew Wyllie and Alastair Currie to show that the second type of cell death was regulated. Finally, they suggested to call this process, apoptosis.[1,2] Apoptosis is a programmed cell death or sometimes cell suicide which plays an important role in a wide variety of normal and pathological processes.[2,3]

Ultraviolet radiation (UVR) is the name given to the light waves that fall between X-rays and visible light rays in the electromagnetic spectrum. This non-ionized and mutagenic light is further divided into three classes: UVA, UVB and UVC.[4,5] UVB has the most penetrating power. The biological effects of UV radiation in various organisms are different.[6]

Dermatophyte fungi are characterized by their ability to grow in keratin and they adapt to their hosts in time. *T. rubrum*, is an anthropophilic fungus causing up to 90% of chronic cases of dermatophytosis.[7-9] This is the reason the dermatopyte *T. rubrum* was selected for this project.

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T. rubrum, which is a dermatophyte fungus, has a positive tropism towards oxygen and negative tropism towards UVB. Therefore, this pathogenic dermatophyte is able to escape from high doses of potentially damaging UVB in vitro.[10]

In this study, we tried to detect the apoptosis feature following the UV-light irradiation in *T. rubrum* and analyze the results in molecular aspect.

Material and Methods

T. rubrum was isolated by using a blunt scalpel and firmly scraping the lesion, particularly at the advancing border, which is a common method for laboratory diagnosis of dermatophyte fungi.

Colonies of *T. rubrum* were inoculated on plates containing Sabouraud Dextrose Broth (SDB) (Difco, East Molesey, U.K.) and kept at room temperature (22°C) for 15 days. The cultured colonies (except the control colonies) were irradiated for 10 min with UV light at a wavelength of 302 nm. The distance between the plates and UV source was 8 cm.

UV lamps of a transilluminator (Upland, CA, USA), which had the maximum quantity of light and the minimum quantity of heat were used as the source of UV light.

The irradiated colonies were divided into three groups and were kept respectively for 1, 24 and 72 hours in a dark chamber at room temperature. Then the mycelia were harvested from the medium by centrifugation and were washed twice with ice-cold sterile phosphate-buffered saline (PBS) and stored at -80°C. The DNA molecules of irradiated and non-irradiated mycelia of *T. rubrum* were isolated. High-molecular-weight DNA from *T. rubrum* was isolated by a modification of the method of Rezaie et al.[9]

Briefly, the harvested mycelia mass was flash-frozen in liquid nitrogen and ground in a fine powder in a porcelain mortar. The mycelia powder was suspended in DNA extraction buffer containing 50 mM Tris-HCl (pH.8), 50 mM EDTA, 3% SDS and 20 µL of Proteinase-K (100 µg/mL). The suspension was then incubated at 65°C for 1 hour and the cellular debris was removed by centrifugation. After addition of 20 µL RNase H (20 U/mL) the suspension was incubated at 37°C for 30 min, extracted once with phenol-chloroform (25:24) and once with chloroform. The DNA was precipitated by addition of an equal volume of 3M sodium acetate (Merck, Darmstadt, Germany), followed by centrifugation. The DNA pellet was rinsed with 70% ethanol and was re-suspended in distilled water.

Finally, 10 µL of DNA of each group was size fractionated in 1% agarose gel containing 5 µL ethidium bromide and was compared with DNA weight marker II of Roche Company.

The observed DNA banding patterns of irradiated colonies were also compared with those of non-irradiated colonies to detect the apoptosis feature.[11]

Results

The DNA molecules of irradiated and non-irradiated *T. rubrum*, which were run through 1% agarose gel did not display any difference in apoptosis in the DNA banding pattern (Figure 1). The bands seen in figure 1 are similar to each other and no differences are observed between them. The procedure was repeated for three times. The weight of the DNA molecules was detected by comparing obtained DNA bands with DNA weight marker II of Roche Company; it was approximately 23.000 bp (Figure 1).

Discussion

Apoptosis is cell death, which occurs in a regulated manner. In other words, death resulting from an organized process involving an internal mechanism that characteristically includes fragmentation of the genome.[4]

Ultraviolet ray is an important physical mutagenic agent, which is usually used in the laboratory to investigate its effects on prokaryotes and eukaryotes. The damaging effect of UVB radiation is very obvious and known to cause skin damages in humans.[12]

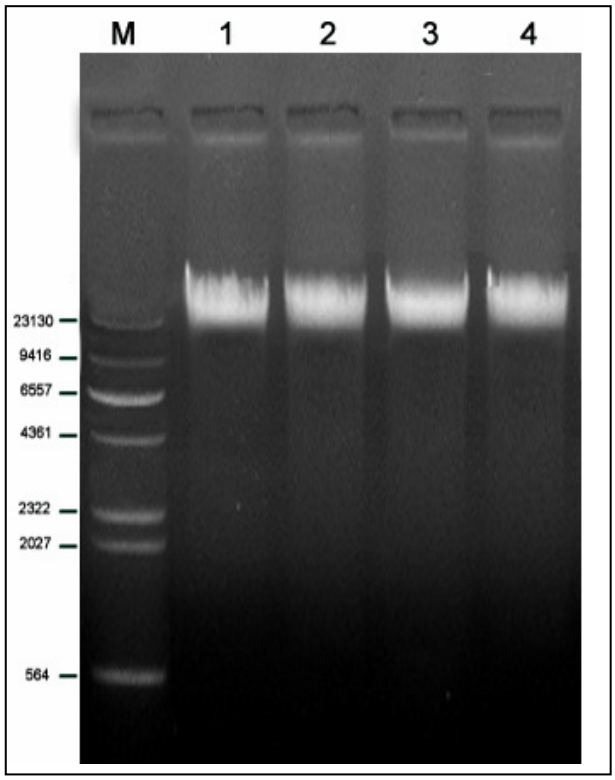

Figure 1. 4 bands of the isolated DNA molecules from control and irradiated colonies of *T. rubrum*, which were run through 1% agarose gel.
Lane 1: DNA weight marker II of Roche Company. The zone of bands is about 23.000 bp.
Lane 2: Isolated DNA molecules from non-irradiated colonies.
Lane 3: Isolated DNA molecules from 10 min-irradiated *T. rubrum* colonies, which were kept for 1 hour in a dark chamber at 22°C after UV light irradiation.
Lane 4: Isolated DNA molecules from 10 min-irradiated *T. rubrum* colonies, which were kept for 24 hours in a dark chamber at 22°C after UV light irradiation.
Lane 5: Isolated DNA molecules from 10 min-irradiated *T. rubrum* colonies, which were kept for 72 hours in a dark chamber at 22°C after UV light irradiation.

UV-radiation is known to inhibit the growth and division of both prokaryotic and eukaryotic cells.[13] UVB-radiation (290-320 nm) depresses DNA, RNA and protein synthesis and inhibits entry into mitosis in cells.[13]

As *T. rubrum* is the most widely disturbed dermatophyte of humans, it was selected for detection of apoptosis feature due to UV-irradiation at the wavelength of 302 nm from a distance of 8 cm.[8]

To prevent the eventual activation of the photoreactivation system, the irradiated colonies of *T. rubrum* were kept in a dark chamber.[12]

UV light may influence moulds in various ways. Depending on the fungal species and the irradiation protocols, various effects such as fungistatic, fungicidal, mutagenic, phototropic and stimulatory effects were described in these organisms.[10]

The host cells can often repair UV-induced DNA damages if they possess the necessary excision repair mechanisms. As in bacteria, the dimer formation and photoreactivation in yeasts, algae and protozoa were characterized.[12]

T. rubrum has a positive tropism towards oxygen and negative tropism towards UVB. Therefore, dermatophyte fungi are able to escape from high doses of potentially damaging UVB in vitro.[10]

Consequently, in this study with the mentioned protocol, no apoptosis feature was detected in DNA banding patterns of UV-irradiated *T. rubrum*. It is possible that the negative tropism of *T. rubrum* towards UVB prevented the formation of apoptosis feature, which needs further investigation.

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