Apoptosis in cutaneous melanomas

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Abstract
Cutaneous melanomas has become one of the most discussed and studied tumor because its particular immunologic development but also its increasing rate worldwide in the last decades. Even thought many patients are diagnosed at an early stage, the death rate continues to rise due to the increasing incidence of more advanced lesions. The aim of this study is to detect apoptosis in 30 cases of cutaneous melanomas using the in situ end-labeling technique (TUNEL) who quantify apoptotic cell death at single cell level and tissues.

Keywords: apoptotic index (AI), cutaneous melanomas.

Introduction
Melanoma is a malignant tumor resulting from the transformation and the proliferation of melanocytes, which derive from neuroectoderm and reside in the basal cell layer of the epidermis. The etiology in melanoma involves genetic susceptibility, a preexistent benign lesion (30% from melanomas) and UV radiation exposure; the risk is increased for individual with light phenotype.

Clinically, cutaneous melanoma appears like a evolutive lesion, frequent unregulated, achromatic or different pigmented; the lesion can develop on normal skin or in the result of evolution from a pigmented benign lesion. From a clinical, histopathological, immunohistochemical and cytogenetic point of view, there were described five evoludional stages: (1) benign pigentary nevus, (2) dysplastic nevus, (3) primary melanoma, radial growth phase, (4) primary melanoma, vertical growth phase, (5) metastatic melanoma.

In the past was considered as a tumor with low frequency, but the recent scientific studies has shown a rate of increase in almost every country and a descent of the age that appears.

The aim of this study is to detect apoptosis in 30 cases of cutaneous melanomas using the in situ end-labeling technique (TUNEL) who quantify apoptotic cell death at single cell level and tissues.

Material and methods
Analysis were performed in 30 paraffin-embedded cases of cutaneous melanomas – 10 cases in situ melanomas (Figures 1 and 2) and 20 cases invasive melanomas(Figures 3 and 4) – from the many cases diagnosed in the Pathology Department of the Dermatology Clinic of Colentina Hospital, Bucharest.

The materials that were used were represented by surgery pieces (cutaneous tumors excised with ontological area around them).

These were manufactured for the histopathological examination (formalin fixed, dehydrated, than included in paraffin and the multiple sections were stained with Hematoxylin–Eosin).

The histopathological examination has shown the following criterions: the histogenetic type, the depth of the tumoral invasion (Clark) and the maximum thickness of the tumor (Breslow), the presence of the ulceration, mitotic rate, the tumoral profile (plans or polypoid lesions), the presence of the melanin and tumor-infiltrating lymphocytes.

The technique for study the apoptotic phenomenon was performed on new 3 micron thick paraffin sections, which are placed on slides and a specific preparation (the DNA is denatured to single-stranded form, and subsequently allowed to hybridize with the Path Vision probes; following hybridization the unbound probe is
removed by a series of washes and the nuclei are counterstained with 4,6-diamino-2-phenylindole).

We had used the in situ end-labeling technique (TUNEL) for quantify apoptotic cell death at single cell level and tissues (Roche). On each histological sample have been studied the fluorescent signal of tumoral cells on 40× magnification.

FISH – fluorescence in situ hybridization involves the precise annealing of a single stranded fluorescently labeled DNA probe to complementary target sequences. This is visible by direct detection using fluorescence microscopy.

Results

Apoptosis or programmed cell death is the most common form of eukaryotic cell death. It is a physiological suicide mechanism that preserves homeostasis, in which cell death naturally occurs during normal tissue turnover [8].

In general, cells undergoing apoptosis display a characteristic pattern of structural changes in nucleus and cytoplasm, including rapid blabbing of plasma membrane and nuclear disintegration; the nuclear collapse is associated with extensive damage to chromatin and DNA-cleavage into oligonucleosomal length DNA fragments after activation of a calcium-dependent endogenous endonuclease [9].

For a good interpretation of the results of the apoptosis study, in all 30 cases of cutaneous melanomas, conventionally, the density and intensity of fluorescent signal was noted: fluorescent signal in many apoptotic tumoral cells +++ (Figure 5), fluorescent signal in apoptotic tumoral cells ++ (Figure 6), fluorescent signal in few apoptotic tumoral cells + (Figure 7), fluorescent signal in very rare apoptotic tumoral cells and absence of the fluorescent signal in apoptotic tumoral cells (Figure 8).

Apoptotic index (AI) was high in the cases of in situ melanomas, but in invasive melanomas (nodular melanomas, superficial spreading melanomas with vertical growth phases, acral lentiginous melanomas, etc.). AI was decreased in tumor cells. We had considered that usually apoptotic cells represent approximately 10% of tumoral cells. In cases in which AI was high, frequently at in situ melanomas, the prognostic is good, when in the others cases in which apoptosis is missing or is very rare in cells the prognostic is reserved.

Discussions

Apoptosis is essential in many physiological processes, including maturation and effector mechanisms of the immune system [1, 5].

In oncology, extensive interest in apoptosis comes from the observation, that this mode of the cell death is triggered by a variety of antitumor drugs, radiation and hyperthermia, and that the intrinsic propensity of tumor cells to respond by apoptosis is modulated by expression of several oncogenes and may be a prognostic marker for cancer treatment [6].

False negative results can be obtained when DNA cleavage can be absent or incomplete in some forms of apoptotic cell death [3]. Sterical hindrance such as extracellular matrix components can prevent access of terminal deoxynucleotidyl transferase to DNA strand breaks. False positive results can be obtained when extensive DNA fragmentation may occur in late stages of necrosis [4]. To confirm apoptotic mode of cell death, the morphology of respective cells should be examined very carefully because it is an important parameter in situations where there is any ambiguity regarding interpretation of results. The main advantage of this simple and rapid procedure is to detect and quantification the apoptosis at a single cell level and make a discrimination of apoptosis from tumor necrosis.

Many regulatory genes have a stimulatory role on oncogenic proliferation or inhibitory – tumoral suppressing genes.

P53 – “the genome guardian” – (oncosuppressor gene) has been shown to be a cellular growth and transformation suppressor, by blocking cell cycle in G1 stage (when DNA can be repaired if an abnormality occurs) as well as an apoptosis stimulator (Figure 10). P53 mutations are the most common seen genetic abnormalities responsible for human cancer development [7].

Its actions can be suppressed either by a mutation that affects the gene reading sense (misses alteration), or by interaction with viral oncoproteins or other cells. “Wild” p53 gene function loss determines abnormalities of cellular cycle control and DNA replications (defections DNA repairing), favoring the growth of certain cells and, subsequently, formation and development of tumoral process.

Because p53 is also a transcription factor, it has been suggested that the mutated variants can interact with various transcription loci determining the high cellular proliferation rate characteristic for neoplasia. P53 mutations can be correlated with DNA alterations by oncogenic factors such as UVB radiation, aflatoxin, oxidative processes. Prognostic value of p53 gene is still unclear. Chen and co have shown that the frequency of mutated p53 is correlated with tumor stage and degree.

Bcl-2 – B cell lymphoma 2 – is the main gene that blocks programmed cellular death (apoptosis). It is found on the mitochondrial membrane or cytoplasm of the progenitor cells with long life and of the epithelium being influenced by growth-factor (Figure 9). Its role is to inhibit the apoptosis induced by growth factor and determine continuous cell proliferation even in the absence of mitotic factors [2].

Conclusions

This non-morphologic technique to evaluate the prognosis of melanoma must be regarded as investigational and cannot be recommended yet for routine application. All necessary pathologic data, such as tumor location, size, depth, ulceration, mitotic activity and growth phase, can be obtained by examination of standard microscopic preparations of melanoma as stained with HE.
Apoptosis in cutaneous melanomas

Figure 1 – Lentigo maligna show hyperpigmentation and slide melanocytic proliferation in the basal cell layer, nest with atypical melanocytes between junction (HE, 10×)

Figure 2 – Superficial spreading melanoma; rounded melanocytes lie singly or nests are scattered throughout epidermis (HE, 20×)

Figure 3 – Acral lentiginous melanoma; nests of atypical melanocytes in dermal-epidermal junction; fascicles of spindle-cells deep in the dermis (HE, 10×)

Figure 4 – Balloon cell melanoma; malignant cells with clear cytoplasm and nuclear atypia (HE, 20×)

Figure 5 – Cutaneous melanoma-FISH: fluorescent signal in many apoptotic tumoral cells +++ (40×)
Figure 6 – Cutaneous melanoma-FISH: fluorescent signal in apoptotic tumoral cells ++ (40×)

Figure 7 – Cutaneous melanoma-FISH: fluorescent signal in few apoptotic tumoral cells + (40×)

Figure 8 – Cutaneous melanoma-FISH: absence of the fluorescent signal in apoptotic tumoral cells (40×)

Figure 9 – Cutaneous melanoma; bcl-2 expression; many cytoplasm show positive reaction (10×)

Figure 10 – Cutaneous melanoma; p-53 expression; many nuclei cells show positive reaction (10×)
FISH have confirmed morphological quantification of apoptosis on cutaneous melanomas. It is important to underline that only the presence of cell proliferation markers is not sufficient and they must be quantified in order to determine their staining markers.

As consequences, we must determine the number of cells, respectively the stained nuclei number per 1000 cells that is then divided to 10 in order to achieve the proliferation index. This operation should be done for several microscopic fields with high cellular density which it is difficult to achieve by free appreciation – simple counting.

Computer should do called IHL the counting with a microscope having an image analyzer with a McIntosh programmed. It is understandable that the results obtained by the two counting methods are different, explaining the possible error of free counting of a large number of cells, as well as of the staining

Accordingly to our study results we concluded that cutaneous melanoma is a tumor with a high growth, having an increased invasion, an unpredictable evolution and generally with an unfavorable prognostic, except those tumors diagnosed in an early phase.

References

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