© PrzypadkiMedyczne.pl, e-ISSN 2084–2708, 2012; 29:118–124
Publikacja dofinansowana przez Ministerstwo Nauki i Szkolnictwa Wyższego

PrzypadkiMedyczne.pl
Wydawnictwo COMVIDEO

Otrzymano: 14–09–2012
Akceptowano: 26–09–2012
Opublikowano: 10–10–2012

Preliminary genetic investigation of the patient with atypical long (55 years) malignant melanoma process

Wstępne badania genetyczne pacjenta o nietypowo długim (55 lat) przebiegu czerniaka złośliwego

Mirosław Dobrut[1], Małgorzata Jaremko[2], Maria Konopacka[3], Jacek Rogoliński[3], Tadeusz Dobosz[2]

[1] Clinic of Oncological Surgery Gliwice, Wybrzeże Armii Krajowej 15, 44–101 Gliwice, PL,
[2] Institute of Forensic Medicine, WMU, ul. Jana Mikulicza–Radeckiego 4, 50–368 Wrocław, PL,
[3] Center of Translational Research and Molecular Biology of Cancer, in: Maria Skłodowska–Curie Memorial Institute of Oncology, Branch in Gliwice, Wybrzeże Armii Krajowej 15, 44-101 Gliwice, PL

Streszczenie

Wstęp: Czerniak złośliwy skóry ma przeważnie dramatyczny przebieg i rokowania zwykle nie są pomyślne. Z tego powodu przypadek pacjenta z 55–letnim przeżyciem po stwierdzeniu wspomnianego nowotworu jest stosunkowo niezwykłym wydarzeniem. Próbując wyjaśnić ten przypadek, zastosowaliśmy test kometowy na limfocytach obwodowych pacjenta w okresie między kolejnymi zabiegami chirurgicznymi, przy napromieniowaniu komórek promieniami γ dawką 2Gy, w porównaniu do dwu innych pacjentów z czerniakiem i czterech osób zdrowych. U obu wspomnianych pacjentów porównawczych z czerniakiem stwierdziliśmy zwiększone względem osób zdrowych wyjściowe uszkodzenia DNA połączone z obniżoną zdolnością naprawczą uszkodzeń. Pacjent, opisywany w tej pracy, co prawda wykazywał większe niż kontrolni pacjenci oraz osoby zdrowe uszkodzeni popromienne materiału genetycznego, ale kinetyka naprawy pęknięć DNA po 180 minutowej inkubacji była u niego wyraźnie wyższa. Zgodnie z hipotezą o związku polimorfizmów genów cytochromu P–450, kodujących enzymy detoksykacyjne, z podatnością na czerniaka, genotypowaliśmy CYP2D6 (*3,*4), CYP2C9 (*2, *3), CYP2C19 (*2) przy użyciu techniki TaqMan, wykazując, że z wyjątkiem CYP2C9*2, wszystkie inne genotypowane loci były heterozygotyczne.

Słowa kluczowe: melanoma malignum, długi czas przeżycia, uszkodzenia i naprawa DNA, test kometowy

Abstract

Background: The skin melanoma is usually connected with rather poor outcome as well as poor overall survival. For that reason the case of melanoma patient X with 55 years long oncological history is unexpected medical phenomenon. In order to explain the basis of such long period of recurrence of the disease with the good outcome in between of next surgeries we compared using comet assay in peripheral blood lymphocytes irradiated in vitro with 2 Gy of–radiation the radiation sensitivity and repair capacity of peripheral blood lymphocytes irradiated in vitro with 2 Gy of γ–radiation, and obtained from patient X, with four healthy donors, and two other melanoma patients. Within the cancer patients group we observed in lymphocytes the higher level of basal damage and decreased DNA repair capacity, than those occurring in controls. Although the initial DNA damage induced by radiation in cells from patient X was high, the kinetic of strand breaks rejoining during the 180 min of incubation was faster than those present in other studied melanoma patients. Following the hypothesis that polymorphism of some cytochrome P–450 enzymes may possibly influence the susceptibility to malignant melanoma, we genotyped polymorphisms of CYP2D6 (*3,*4), CYP2C9 (*2, *3), CYP2C19 (*2) by TaqMan technology in DNA obtained from patient X. With the exception of CYP2C9*2, we determined all of the genotypes as heterozygote.

key words: melanoma malignum, ultra–late recurrence, DNA damage and repair, comet assay

Adres pocztowy: Jacek Rogoliński, Centrum Badań Translacyjnych i Biologii Molekularnej Nowotworów, Wybrzeże Armii Krajowej 15, 44–101 Gliwice, e–mail: rogolinski@io.gliwice.pl

PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl
PrzypadkiMedyczne.pl

PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl

Introduction

Melanoma is an aggressive skin neoplasma, usually connected with rather poor prognosis. Survival is routinely predicted on the basis of tumor thickness. However, additional prognostic indicators have been identified, including patient sex and age, anatomical site of the primary tumor, mitotic rate of the tumor, presence of ulceration, microscopic satellites, or tumor–infiltrating lymphocytes, tumor regression or angiogenesis, and the Clark level of invasion [1, 2, 3, 4, 5].

Predisposition to melanoma is genetically heterogeneous. The susceptibility to disease may be associated with polymorphisms of genes responsible for DNA repair, cell growth and differentiation, and detoxification of xenobiotics [6]. Several candidate genes have been reported likely to predispose to sporadic melanoma, including GSTM1, MC1R, EGF, and CYP2D6 [7]. The CYP2D6 poor metabolizer genotype has been associated with increased risk of disease [8]. Some studies suggested that CYP2D6 genotype may also influence the outcome of patients with malignant melanoma [9].

According to current studies the majority of cancers arise as a consequence of interplay between exposure to environmental agents that are toxic or mutant and genetic factors. The higher organisms have developed complex mechanism for detoxification of the xenobiotics. However, one of the possible pathways in this mechanism may be metabolic activation of procarcinogens, which lead to carcinogenesis. The cytochrome P450 is one of the best characterized enzyme systems, and plays a significant role in the metabolism of environmental and occupational exposures [10]. Several enzymes from this family are encoded by a number of polymorphic alleles. The resulting variation in activity of these enzymes has been suggested to modify the risk of developing a number of malignant diseases. Polymorphic cytochrome P450 enzymes include among other CYP2D6, CYP2C9 and CYP2C19 [11, 12]. For all of them, individuals are phenotyped as poor metabolizers (PM), intermediate metabolizers (IM) or extensive metabolizers (EM) depending upon the alleles inherited [13, 14, 15, 16]. Moreover the different type of phenotype may influence cancer susceptibility: PM through the lower activity to detoxify particular xenobiotics, and EM through higher activation of procarcinogens to DNA – damaging intermediates [11]. For instance, the CYP2D6 EM phenotype could increase risk for bladder cancer and hepatocellular carcinoma [17, 18], whereas PM phenotype for oral cancer and astrocytoma [19, 20].

In Caucasians a PM phenotype for CYP2D6 includes inheritance of three mutant alleles: CYP2D6*3, *4 and *5 [11, 21]. CYP2D6*4 is the most frequent null allele, and represents 75% of mutant alleles [14, 22], whereas CYP2D6*3 only 5% ([11]. It was determined that homozygosity for the mutant alleles, CYP2D6*4 and CYP2D6*3, is likely associated with susceptibility and outcome in malignant melanoma [9].

For CYP2C19, the 88% of the Caucasians, who are homozygote mutants for the CYP2C19*2 allele and represent PM phenotype [11, 22, 23, 24]. In case of CYP2C9, the most common variant allele in Caucasians is CYP2C9*2 [22]. The occurrence either CYP2C9*2 or CYP2C9*3 allele reduce activity of this enzyme [25, 26, 27].

A repair capacity of DNA damage plays also an important role against different genotoxic factors leading to mutation and carcinogenesis. Single cell gel electrophoresis (comet) assay is a sensitive method for evaluation of DNA damage and kinetic of its repair and it is widely used in radiobiological studies. This assay detects single and double strand breaks and also the capacity of individual cells to repair of the damage induced by ionizing radiation [28].

The aim of this study was to genotype drug metabolizing enzymes by TaqMan technology, as well as to measure repair capacity of radiation– induced DNA damages by comet assay, using DNA obtained from skin melanoma patient X with unusual 55 years long oncological history.

PrzypadkiMedyczne.pl

Description of the case

Patients

The blood samples were collected from Polish Caucasian at the Department of Experimental and Clinical Radiobiology of Oncology Center in Gliwice (Poland). The subjects consisted of four healthy controls including 2 female and 2 male (all of them non–smokers, 26–50 years), two skin melanoma patients (male, 58–63 years), and skin melanoma patient X (male, 71 years) with 55–years oncological history, described in tab. 1. All tumors were histological diagnosed at the Clinic of Oncological Surgery as invasive malignant melanoma. The following pathological data were recorded: Breslow thickness, Clarck classification and tumor site. The controls comprised randomly recruited subjects with Mel. malignum and without clinical or histological evidence of malignancy.

DNA preparation

DNA was extracted using classic organic (phenol) extraction according to Kunkel et al. [29].

Cell culture and irradiation

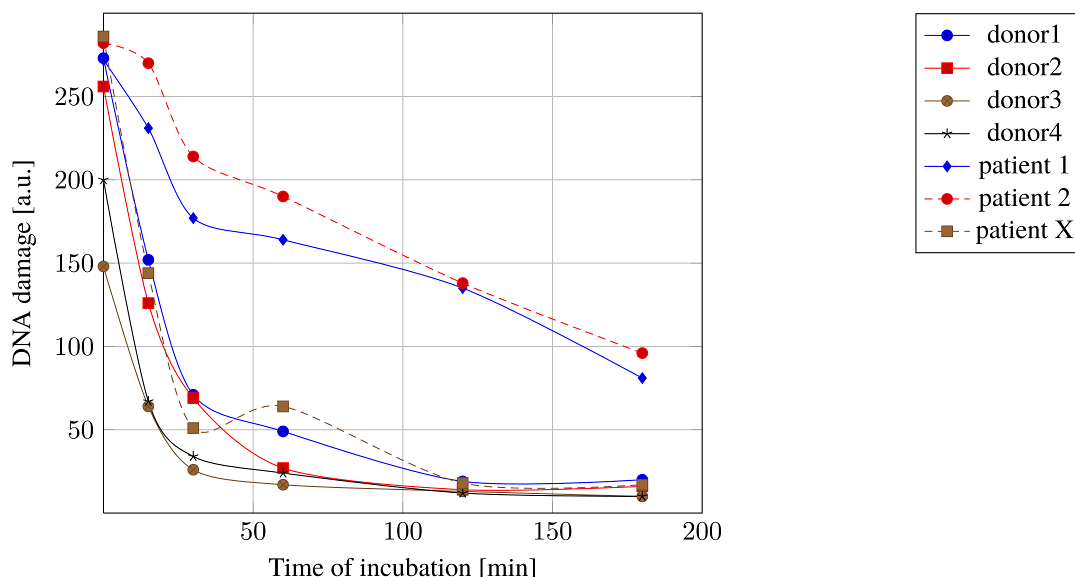
Whole blood (2 ml) samples were collected from subjects. Duplicate cultures of leukocytes were prepared by adding 100 μl of blood to 500 μl of RPMI medium (Sigma) supplemented with 15% foetal bovine serum (ICN, Biomedicals) and antibiotics.

The γ–ray irradiation was carried out with a 60 Co Gammatron (Siemens) at dose of 2 Gy (the dose–rate 1 Gy/min.) on ice.

Tablica 1: Patient X oncological history

Date	Localization	Diagnosis	Therapy
23.IX.1957	face	Ca. basocellulare	radiotherapia (5.000 R)
29.IX.1966	face	Ca. basocellulare	radiotherapia (5.000 R)
06.VI.1967	face	Ca. plano keratodes	radiotherapia (6.000 R)
1968	face	Ca. plano	excisio
19.XII.1987	nape	Ca. basocellulare	excisio
1992	arm	Ca. basocellulare	excisio
1995	arm	Mel. malignum, III Clark	excisio
18.VII.1996	scapula	Mel. malignum, metastaticum	excisio
18.VII.1996	trunk	Mel. malignum, metastaticum	excisio
26.IX.1996	arm	Mel. malignum, metastaticum	excisio
28.IX.1996	trunk	Mel. malignum, metastaticum	excisio
15.I.1997	abdomen	Mel. malignum, II Clark	excisio
15.I.1997	trunk	Mel. malignum, I Clark	excisio
21.V.1997	arm and trunk	Mel. malignum, I-III Clark	excisio
30.VI.1997	hand	Keratoacanthoma	excisio
14.IX.1997	trunk	Mel. malignum, II Clark	excisio
14.IX.1997	trunk	Mel. malignum, III Clark	excisio
06.X.1997	trunk	Mel. malignum, metastaticum	excisio
06.X.1997	trunk	Mel. malignum, II Clark	excisio
16.I.1998	trunk	Mel. malignum, III Clark	excisio
31.VII.1998	trunk	Mel. malignum, III Clark	excisio
04.IX.1998	arm and trunk	Keratoacanthoma	excisio
02.VII.1999	head	Ca. basocellulare	excisio
07.IV.2000	foot	Ca. plano keratodes G1	excisio
05.V.2000	trunk	Mel. malignum, III Clark	excisio
02.II.2001	trunk	Mel. malignum, III Clark	excisio
22.VII.2002	leg	Naevus pigmentosus epidermo-dermalis	excisio
20.V.2003	hand	Ca. basocellulare	excisio
28.VII.2003	head	Mel. malignum in naevo	excisio
14.XI.2003	trunk	Mel. malignum in naevo I Clark	excisio
16.I.2004	trunk	Naevus melanocyticus compositus	excisio completa
24.II.2004	forearm	Keratoacanthoma	excisio tumoris
12.IV.2011	arm	Mel. malignum	excisio
21.X.2011	arm	Mel. malignum, metastasis	excisio
02.XII.2011	trunk	Mel. malignum, metastasicum	excisio

PrzypadkiMedyczne.pl



Rysunek 1: Comparison of DNA repair kinetics in human lymphocytes of healthy donors and melanoma patients

PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl

PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl

Comet assay

Spontaneous and radiation–induced DNA strand breaks were determined using the single cell gel electrophoresis (comet assay) in alkaline conditions [30] in order to compare radiation–induced DNA damage and repair capacity in peripheral blood lymphocytes between patient X, two melanoma patients and four healthy donors.

Samples poured into plastic dishes were irradiated on ice and next incubated for 0, 15, 30, 60, 120 and 180 min. at 37°C. The repair incubation was stopped by placing the samples into an ice bath. The cells were suspended in 1% low–point–melting agarose (Sigma), and placed on microscope slides. Afterwards the cells were lysed for 60 min. in buffer containing 2.5 M NaCl, 100 mM EDTA–Na2, 10 mM Tris–HCl (pH 10) and 1% Triton X–100. After lysis the slides were placed in an electrophoresis tank and immersed in 300 mM NaOH and 1 mM EDTA–Na2 buffer (pH 13) for 30 min. Then electrophoresis was conducted in the same buffer for 20 min. at 1 V/cm. Then slides were washed 3 times in 0.4 M Tris–HCl (pH 7.5) and stained with ethidium bromide. Analysis of DNA damage was performed using a fluorescence microscope (Zeiss, Opton) with 450 nm excitation and 520 nm emission filters. One hundred cell (comets) of DNA damage were measured according to scale from 0 (no visible tail) to 4 (head small, most DNA in long tail) [31].

TaqMan assays

We genotyped DNA of patient X using the TaqMan ABI PRISM™ 7900HT Sequence Detector (Applied Biosystems).

Allelic discrimination (AD) was performed for the following SNPs: CYP2D6*3 (del_2637_A>0), CYP2D6*4 (1934_G>A); CYP2C9*2 (430_C>T), CYP2C9*3 (1075_A>C), CYP2C19*2 (681_G>A) using TaqMan®Pre–Developed Assay Reagents for Allelic Discrimination (PDAR, Applied Biosystems) listed in tab 2.

Tablica 2: PDAR commercial kits used for AD assays

Locus	Assay kit (part number)
CYP2D6*3	4312554
CYP2D6*4	4312555
CYP2C9*2	4312559
CYP2C9*3	4312560
CYP2C19*2	4312561

Reactions was carried out using a 96–well tray and optical caps (PE Applied Biosystems) with a 20–µl final reaction mixture containing 12,5µl of TaqMan Universal PCR Master Mix (2x TM uMM buffer), 2,5 µl PDAR AD OligoMix, 5 µl DNA, and 5 µl purified water under following conditions 30 cycles of amplification and each cycle included 95°C denaturation for 15 sec, 61°C annealing/extension for 1 min.

The fluorescence signal was monitored on–line using the laser detector of the ABI Prism 7900 Sequence Detection System (Applied Biosystems). To avoid any contamination during the DNA extraction and the PCR set–up all steps were performed in a separate laboratory under sterile condition. In every run four negative controls (purified water) were included to exclude false–indication of alleles.

Results

Comet assay

The obtained results of the basal DNA damage detected by comet assay in lymphocytes from healthy donors and cancer patients are presented in tab. 3

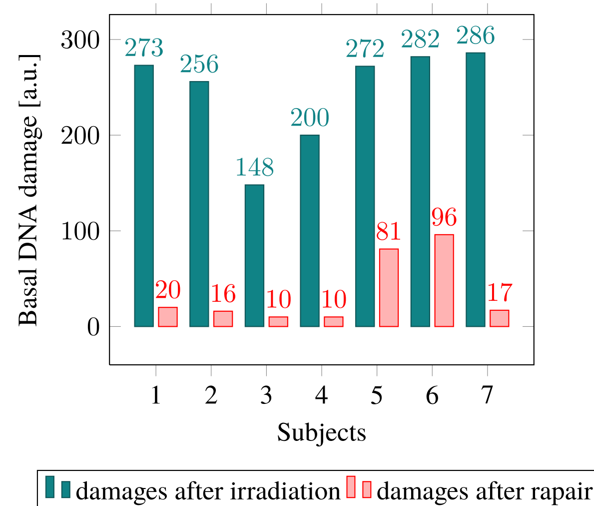
Tablica 3: The background of DNA damage detected by the comet assay in human lymphocytes from healthy donors and melanoma patients

Probe	Basal damage [a.u.]
Donor 1	17
Donor 2	20
Donor 3	12
Donor 4	9
Patient 1	39
Patient 2	71
Patient X	13

PrzypadkiMedyczne.pl

The initial amount of DNA breaks induced by γ–radiation and kinetics of its rejoining are presented in Fig. 1. Although the initial DNA damage induced by radiation (time 0) is comparable in all tested samples, the kinetics of DNA breaks rejoining were slower in cells of 1st and 2nd melanoma patients cells than in control cells.

There were still over 60% unrepaired damages in lymphocytes of cancer patients after 60 min. of incubation, whereas only about 25% and 20% in cells collected from patient X, and controls, respectively. With the exception of patient X, the residual damage that persisted after 180 min. of incubation was higher in the lymphocytes of melanoma patients than in control donors.



Rysunek 2: The level of DNA damages detected by the comet assay in human lymphocytes from healthy donors and melanoma patients, 1–4 healthy donors, 5–6 melanoma patients, 7– patient X

PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl

In Fig. 2 we compared the initial level of DNA damage detected immediately after irradiation with the level of DNA damage measured after repair in lymphocytes from healthy donors and melanoma patients. The highest initial DNA damage were exhibited by patient X. However, the repair capacity by this patient was similar to those in healthy donors. The two other melanoma patients presented the lowest capacity in DNA repair.

We have shown that cells of two melanoma patients were characterized by higher level of background damage in comparison with the control donors, whereas the level of DNA basal damage measured in lymphocytes from patient X was comparable with values obtained from healthy donors.

Genotyping

We successfully genotyped polymorphism of drug metabolizing enzymes, and the results of obtained genotype are listed in tab 4. The negative controls stayed false in each set of reaction.

Tablica 4: Genotype obtained for patients X by allelic discrimination

Locus	Genotype occurring in patient X
CYP2D6*3 (del_2637_A>0)	A/delA
CYP2D6*4 (1934_G>A)	G /A
CYP2C9*2 (430_C>T)	C/C
CYP2C9*3 (1075_A>C)	A/C
CYP2C19*2 (681_G>A)	G/A

We identified that the patient X is heterozygote for the following alleles: CYP2D6*3, CYP2D6*4, CYP2C9*3, and CYP2C19*2. For CYP2C9*3 he presented homozygous wild type genotype.

The mutant alleles are associated with deficient activity of enzymes, and carriers of two nonfunctional alleles are phenotypically poor metabolizers because they have a severely impaired metabolism of substrates.

In our case, heterozygous genotype may represent the intermediate metabolizer phenotype. However, we are not able to specify the type of phenotype. In the Polish population the published frequency of CYP2D6*3 and CYP2D6*4 were 1.3% and 23.1%, respectively. The frequency for the wild type CYP2D6*1 was 75.7% [32]. The frequencies of poor metabolizers for CYP2D6 nulled genotype among a Polish population were similar to those observed in other Caucasian populations [33].

A significant increase in the proportion of poor metabolizers or heterozygotes was seen in melanoma and some other cancer patients. This could be explained by a role for CYP2D6 in carcinogen detoxification or by linkage to another cancer–causing gene [34].

Currently, there is no data available about the genotype frequencies of CYP2C9 and CYP2C19 in a Polish population.

Discussion

During the past several decades, we have learned that melanoma is a heterogeneous disease with wide variation in survival, a finding that reflects several well–defined clinical and pathologic prognostic factors. Malignant melanoma is the leading cause of death from skin diseases, and its mortality is increasing faster than for any other malignant disease except lung cancer [7]. In our work we have presented unusual case of melanoma patient X with 55 years oncological history.

This medical phenomenon is characterized by very long– term survival with randomly repeated metastases to different part of skin during whole period of disease. Since time of the first diagnosis, patient X has undergone several surgeries, and three roentgenotherapies. There was not applied any chemotherapy treatment. Although, such often recurrence of the disease the patient X survive until today.

Routinely, the occurrence of such long term follow–up is atypical for malignant melanoma patients, because the longest time of survival may be about 10 years, and in the event of metastases even shorter. Although most melanomas recur within 10 years of primary diagnosis, several reports presented some cases with recurrence of cutaneous malignant melanoma after more than 15 years [35, 36] and more than 20 years: 26 years [37] and 29 years [38, 39].

In case of patient X we have suspected exhibition of a very high capacity for DNA repair. The initial DNA damages in the cells were not so high, and we detected by comet assay that kinetic of strand breaks rejoining was faster than this present in other melanoma patients, and comparable with controls. Correct repair of DNA damage plays a protective role against genotoxic factors, while its impairments leads to mutation and may be basis of carcinogenesis. Single cell electrophoresis (comet assay) is sensitive method for evaluation of DNA damage and kinetics of its repair. Our results from the comet assay indicate a great variation in response to ionizing radiation in lymphocytes of tested subjects. However, the lymphocytes of patient X were characterized by high repair potential.

This could enable patient X to avoid accumulation of mutations caused by radiation. It seems that the patient X demonstrates not so high susceptibility to radiation induced changes in nucleic acids. For that reason the repair system is able to remove more easily the DNA damages, and this could be a key to elucidation of such long–term survival period connected with good outcome between the next surgeries.

Individual capacity if removal of DNA damage induced by ionizing radiation seems to be important factor responsible for survival, but not for forming of metastases. This suggestion require analysis of more number of melanoma patients.

Bibliografia

1. Zettersten E., Shaikh L., Ramirez R. et al. Prognostic factors in primary cutaneous melanoma. *Surg Clin North Am*, 2003, 83:61–75.

PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl

2. Jain B.P. Prognostic Model for Primary Melanoma. *Ann Intern Med*, May 1997, 126(10):832.
3. Balch C.M., Murad T.M., Soong S.J. et al. A multifactorial analysis of melanoma: prognostic histopathological features comparing Clark's and Breslow's staging methods. *Ann Surg*, 1978, 188(6):732–742.
4. Brenner B., Borok S., Rakowsky E. et al. Older age and second skin cancer as prognostic factors in localized malignant melanomas. *Oncol Rep*, 2003, 10:2051–7.
5. Day C.L. Jr, Mihm M.C. Jr, Lew R.A. et al. Prognostic factors for patients with clinical stage I melanoma of intermediate thickness (1.51–3.99 mm). A conceptual model for tumor growth and metastasis. *Ann Surg*, 1982, 195:35–43.
6. Hayward N.K. Genetics of melanoma predisposition. *Oncogene*, 2003, 22:3053–62.
7. Meyer P., Sergi C., Garbe C. Polymorphisms of the BRAF gene predispose males to malignant melanoma. *J Carcinog*, 2003, 2:7.
8. Hutchinson P.E., Osborne J.E., Lear J.T. et al. Vitamin D receptor Polymorphisms are Associated with Altered Prognosis in Patients with Malignant Melanoma. *Clin Cancer Res*, 2000, 6(2):498–504.
9. Strange R.C., Ellison T., Ichii-Jones F. et al. Cytochrome P450 CYP2D6 genotypes: association with hair colour, Breslow thickness and melanocyte stimulating hormone receptor alleles in patients with malignant melanoma. *Pharmacogenetics*, 1999, 9(3):269–276.
10. Smith G., Stanley L.A., Sim E. et al. Metabolic polymorphisms and cancer susceptibility. *Cancer Surv*, 1995, 25:27–65.
11. Roddam P.L., Rollinson S., Kane E. et al. Poor metabolizers at the cytochrome P450 2D6 and 2C19 loci are at increased risk of developing adult acute leukemia. *Pharmacogenetics*, 2000, 10(7):605–15.
12. Stubbins M.J., Harries L.W., Smith G. et al. Genetic analysis of the human cytochrome P450 CYP2C9 locus. *Pharmacogenetics*, 1996, 6:429–39.
13. Dresser G.K., Bailey D.G. A basic conceptual and practical overview of interactions with highly prescribed drugs. *Can J Clin Pharmacol*, 2002, 9:191–98.
14. Marez D., Legrand M., Sabbagh N. et al. Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. *Pharmacogenetics*, 1997, 7(3):193–202.
15. Raimundo S., Fischer J., Eichelbaum M. et al. Elucidation of the genetic basis of the common 'intermediate metabolizer' phenotype for drug oxidation by CYP2D6. *Pharmacogenetics*, 2000, 10(7):577–81.
16. Zanger U.M., Fischer J., Raimundo S. et al. Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. *Pharmacogenetics*, 2001, 11(7):573–85.
17. Brockmöller J., Cascorbi I., Kerb R. et al. Combined analysis of inherited polymorphisms in arylamine N–acetyltransferase 2, glutathione S–transferases M1 and T1, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder cancer risk. *Cancer Res*, 1996, 56:3915–25.
18. Agúndez J.A., Olivera M., Ladero J.M. et al. Increased risk for hepatocellular carcinoma in NAT2–slow acetylators and CYP2D6– rapid metabolizers. *Pharmacogenetics*, 1996, 6(6):501–12.
19. Worrall S.F., Corrigan M., High A. et al. Susceptibility and outcome in oral cancer: preliminary data showing an association with polymorphism in cytochrome P450 CYP2D6. *Pharmacogenetics*, 1998, 8(5):433–9.
20. Elexpuru-Camiruaga J., Buxton N., Kandula V. et al. Susceptibility to astrocytoma and meningioma: influence of allelism at glutathione S- transferase (GSTT1 and GSTM1) and cytochrome P–450 (CYP2D6) loci. *Cancer Res*, 1995, 55(19):4237–39.
21. Leathart J.B., London S.J., Steward A. et al. CYP2D6 phenotype – genotype relationships in African–Americans and Caucasians in Los Angeles. *Pharmacogenetics*, 1998, 8(6):529–41.
22. Meyer U.A., Zanger U.M. Molecular mechanisms of genetic polymorphisms of drug metabolism. *Annu Rev Pharmacol Toxicol*, 1997, 37:269–96.
23. Wedlund P.J. The CYP2C19 Enzyme Polymorphism. *Pharmacology*, 2000, 61:174–83.
24. De Morais S.M., Wilkinson G.R., Blaisdell J. et al. Identification of a New Genetic Defect Responsible for the Polymorphism of (S)– Mephenytoin Metabolism in Japanese. *Mol Pharmacol*, 1994, 46(4):594–8.
25. London S.J., Sullivan-Klose T., Daly A.K. et al. Lung cancer risk in relation to the CYP2C9 genetic polymorphism among Caucasians in Los Angeles County. *Pharmacogenetics*, 1997, 7:401–4.
26. Crespi C.L., Miller V.P. The R144C change in the CYP2C9*2 allele alters interaction of the cytochrome P450 with NADPH: cytochrome P450 oxidoreductase. *Pharmacogenetics*, 1997, 7:203–10.
27. Gill H.J., Tjia J.F., Kitteringham N.R. et al. The effect of genetic polymorphisms in CYP2C9 on sulphamethoxazole N–hydroxylation. *Pharmacogenetics*, 1999, 9(1):43–53.
28. Müller W.U., Bauch T., Stüben G. et al. Radiation sensitivity of lymphocytes from healthy individuals and cancer patients as measured by the comet assay. *Radiat Environ Biophys*, 2001, 40:83–9.
29. Kunkel L.M., Smith K.D., Boyer S.H. et al. Analysis of human Y–chromosome-specific reiterated DNA in chromosome variants. *Proc Natl Acad Sci U S A*, 1977, 74(3):1245–9.
30. Singh N.P., McCoy M.T., Tice R.R. et al. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res*, 1988, 175:184–91.
31. Gedik C.M., Ewen S.W., Collins A.R. Single–cell gel electrophoresis applied to the analysis of UV–C damage and its repair in human cells. *Int J Radiat Biol*, 1992, 62:313–20.
32. Niewinski P., Orzechowska-Juzwenko K., Hurkacz M. et al. CYP2D6 extensive, intermediate, and poor phenotypes and genotypes in a Polish population. *Eur J Clin Pharmacol*, 2002, 58(8):533–5.
33. Gawronska-Szklarz B., Wójcicki M., Kuprianowicz A. et al. CYP2D6 and GSTM1 genotypes in a Polish population. *Eur J Clin Pharmacol*, 1999, 55(5):389–92.
34. Wolf C.R., Smith C.A., Gough A.C. et al. Relationship between the debrisoquine hydroxylase polymorphism and cancer susceptibility. *Carcinogenesis*, 1992, 13(6):1035–8.
35. Gamel J.W., George S.L., Edwards M.J. et al. The long–term clinical course of patients with cutaneous melanoma. *Cancer*, 2002, 95:1286–93.
36. Nozaki M., Fukuda R., Kawashima M. et al. A Case of a Malignant Melanoma with Late Metastases 16 Years After the Initial Surgery. *Jpn J Clin Oncol*, 1999,

PrzypadkiMedyczne.pl

PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl © PrzypadkiMedyczne.pl

29(2):109–11.

37. Briele H.A., Beattie C.W., Ronan S.G. et al. Late recurrence of cutaneous melanoma. *Arch Surg*, 1983, 118:800–3.

38. Tsao H., Cosimi A.B., Sober A.J. et al. Ultra–late recurrence (15 years or longer) of cutaneous melanoma. *Cancer*, 1997, 79:2361–70.

39. Bredgaard R., Lock-Andersen J. Recurrence of cutaneous malignant melanoma after more than 20 years. *J Plast Surg Hand Surg*, 2011, 45:113–6.

Komentarz:

Autorzy artykułu przedstawiają przypadek czerniaka złośliwego skóry, który przeżył 55 lat od jego rozpoznania mimo kilkakrotonych nawrotów i przerzutów. Jedną z przyczyn rozwoju nowotworu jest utrata zdolności do naprawy DNA, wobec powyższego postanowili udowodnić postawioną przez siebie hipotezę, że w opisywanym przypadku procesy naprawcze zostały przyspieszone przez stosowane w leczeniu napromieniowanie promieniami γ. Dla porównania wykonano badania u innych pacjentów z rozpoznaniem czerniaka oraz pacjentów zdrowych i wykazali istotne przyspieszenie procesów naprawczych u pacjenta z 55-letnim przeżyciem. Pracę uważam za niezwykle interesującą i wartą publikacji, nie można jednak na podstawie przeprowadzonych przez Autorów badań, przeprowadzonych u jednego pacjenta, wyciągać zbyt daleko idących wniosków, powinny one być kontynuowane.

prof. dr hab. Michał Jeleń

PrzypadkiMedyczne.pl