ORIGINAL ARTICLE

Mutations in Tumor Suppressor Gene NBS1 in Adult Patients with Malignancies

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SUMMARY

Background. Mutations 657del5 and R215W in exon 6 of tumor suppressor gene NBS1 are found in 1% of Slavic populations. Increased occurrence of cancer was repeatedly reported in adult relatives of patients with Nijmegen breakage syndrome. Nearby significantly increased frequency of NBS1 heterozygotes was found among child patients with tomurs, which seems not to play any important role in cancerogenesis in childhood. However, the proportion of NBS heterozygotes among adult patients with malignancies could be significant, and their therapy and follow up should respect their hyperradiosensitivity.

Methods and Results. Mutations in exon were studied in 706 adult patients with malignancies. We found 5 NBS heterozygotes, which is not more than the population prevalence (1:129–165). Increased frequency of NBS heterozygotes, though non-significant, was found among patients with colorectal cancer (2/101), breast cancer (1/60), skin malignancies and melanomas (1/98). Comparison of frequency of NBS heterozygotes among tumor patients with the incidence of NBS heterozygotes among individuals older than 70 years shows significant difference.

Conclusions. Surprisingly, only one NBS heterozygote was found among 228 patients with non-Hodgkin's lymphoma, the malignancy that is a common and often lethal complication in NBS homozygotes. Other types of malignancies were uncommon, and only one R215W heterozygote was found. Identification of heterozygotes among their children and other relatives of patients with malignancy that are carriers of mutation offers possibility of efficient prevention against malignancies by avoidance from ionization mutagenes.

Key words: tumor suppressor gene NBS1, Slavic mutation 657del5 and R215W in exon 6, NBS heterozygotes among adult patients with malignancies.

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Nijmegen breakage syndrome (NBS) is a deficient DNA reparation (inherited in an autosomal recessive manner) with hyperradiosenzitivity a high occurrence of lymphoretikular malignancy, which is detected mostly in Slavic populations (1–3). All NBS patients with Slavic ancestors were homozygotes or compound heterozygotes of 5bp deletion in exon 6 (mutation 657del5) (1,2) to date.

The frequency of heterozygotes of mutation 657del5 found in our population is 0.5 – 1 percent (4), and the frequency of heterozygotes of R215W detected in our country is 1:234 (5). Genealogy of patients with NBS provided evidence of increased occurrence of malignancies in heterozygotes in middle or older age (6, 7) and both heterozygotes of another chromosomal instability and hyperradiosensitivity syndrome - Louis- Bar's ataxia telangiectasia. Nibrin, a protein product of *NBS1* gene, is a part of the MRE11/RAD50 complex similar to 350-kDA protein product of *ATM* gene, which is responsible for ataxia telangiectasia (2). This complex is responsible for DNA repairs (double strand breaks-DSBs), and that is why carriers of mutations in *NBS1 and ATM* genes have insufficient reparation of chromosomal spontaneous breaks, but mainly of aberrations, induced by radiation.

Protection of heterozygotes of tumor suppressor gene *NBS1* against ionising radiation is viewed as an effective prevention of malignancy development. Detection of heterozygotes in families of patients with Nijmegen breakage syndrome and among oncological patients and their relatives is important for individual therapy and prevention of secondary tumors.

The aim of this study is to find out the occurrence of heterozygotes of mutations 657del5 and R215W among adult patients with malignancies in comparison with occurrence of heterozygotes among newborns and individuals older than 70 years (10, 11).

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Tab. 1. Types of malignancies in adult patients

Tumor	number of patients	sex ratio	M:F age over 50 years
colorectal carcinoma	101	54:47	83
non-Hodgkinęs lymphoma	228	anonymous	specimens
skin carcinoma and melanoma	98	41:57	72
breast carcinoma	60	women only	42
urinary bladder carcinoma	38	26:12	30
thyroidal carcinoma	22	10:12	18
lung carcinoma	26	15:11	21
other solid malignant tumors (gastric, uterine, renal, prostate, cerebral, connective tissue)	133	68:65	97
total	706	214:264 (+ 228 anonymous)	363

COHORT OF PATIENTS AND METHODS USED

We collected dry blood spots on screening papers from 706 patients treated for different types of malignancies primarily at the departments of Charles University 2nd Faculty of Medicine and Faculty Hospital Motol (Prague) during the years 2000-2004. Table 1 shows the number of assessed patients with particular tumor types.

Most patients were older then 50 years, but it is not possible to establish either mean age or median because of 228 anonymous specimens of patients with NHL. The sex ratio was not significantly shifted as compared with 1:1. It cannot be specified with respect to anonymous specimens of patients with NHL.

Detection of mutation 657del5 was performed in Prague and detection of mutation R215W in Berlin. Methods used did not differ between the two locations. A piece size 2 mm in diameter was separated from each blood spot and used for mutation analysis by means of PCR-SSCP (polymerase chain reaction-single strand conformation polymorphism) in PCR reaction with primers marked with fluorescence specific for exon 6 of *NBS1* gene, as has repeatedly been described (7, 11). All specimens where aberrant shift was detected were subsequently sequenced, and in this way the second mutation R215W was proved.

RESULTS

The occurrence of *NBS1* heterozygotes of mutations 657del5 and R215W in exon 6 in particular groups of patients with malignancies is showed in Table 2.

The expected occurrence of heterozygotes would be according to the population data (1:128–165 among newborns) in cohort of 706 patients with malignancies 4.2-5.4 persons. The difference between occurrence of 5 heterozygotes of mutation 657del5 among 706 adult patients with malignancy and the occurrence of heterozygotes in population of newborns is not significant (chi-square –test); it is the same if we divide the cohort according to tumor types. Frequency of heterozygotes of mutation 657del5 in *NBS1* gene increases in comparison with the expected count according to the population data in groups of patients with colorectal tumor on the margin of significance and non-significantly increased in the group of patients with breast cancer and skin tumors.

However, if we compare occurrence of NBS heterozygotes among patients with malignancy and population of individuals at the age of 70 and older, (1:423), the difference would be significant, also in comparison with the occurrence in the whole cohort of adult patients older than 50 years.

DISCUSSION

Increased frequency of malignant tumors among relatives of patients with NBS and AT which were carriers of germinal mutations in v *ATM* a *NBS1* genes has repeatedly been described (6, 8, 9, 12, 13).

Frequency of NBS heterozygotes in populations that are decreasing with the increasing age could show loss of NBS heterozygotes due to the death caused by malignancy (10, 11). This finding and easy identification of mutation with predominant occurrence in Slavic population (2) in NBS gene led to study of heterozygotes frequency among patients with malignancy and also in tumor tissues (14) of heterozygotes of germinal mutations. Steffen et al. found significantly more mutations 657del5 and R215W than would correspond with occurrence in population in the group of more than 1000 oncology patients in the year 2004 (13). Significantly increased occurrence was detected mainly in groups of patients with melanoma, breast carcino-

Tab. 2. NBS heterozygotes between patients with malignancy

Tumor mutation	number of patients	number of NBS heterozygotes 657del5	R215W	frequency 657del5	R215W
colorectal carcinoma	101	2	0	1:50	
NHL	228	1	not assessed	1:228	
skin carcinoma and melanoma	98	1	0	1:98	
breast carcinoma	60	1	0	1:60	
others	224	0	1		1:224
total	706	5	1	1:141	1:483

Tab. 3. Characteristics of heterozygotes of mutations 657del5 and R215W in the group of patients with malignancy

Patient	sex	age (years)	tumor type
MB	F	71	colorectal carcinoma
VB	M	65	colorectal carcinoma
VŠ	F	63	breast carcinoma
XX	?	?	NHL
MS	M	43	melanoma
MŽ	F	30	m. Hodgkin

MB to MŽ – IDC of patients, F– female, M– male, NHL – non Hodgkin's lymphoma

ma, colorectal carcinoma and NHL. *NBS1* gene was confirmed as tumor suppressor gene (14, 15) by verification of loss of heterozygosity (LOH) or by detection of gene amplification in tumor tissue. The results of our smaller cohort of adult patients with malignancy correspond with these results as far as germline mutation 657del is concerned, with the exception of patients with NHL that was detected by Souček et al (16). We found only one heterozygote of mutation R215W among 483 assessed patients with malignity, although the frequency of this mutation found in the population of newborns was 1:234 and the expected number would be 2. The occurrence of this mutation in a cohort of Polish oncology patients was significantly increased (13). The difference could be explained by the small number of patients in the cohort, particularly after its division according to particular tumor types.

The occurrence of heterozygotes of both mutations in *NBS1* gene – 657del5 and R215W – is 1 percent in Slavic population (2, 4). Also the medium-increased risk of malignancies in these hyperradiosensitive individuals can thereafter comprise development of many new malignancies every per year.

DNA hyperradiosensitivity of carriers of mutation in *NBS1* gene offers the possibility of efficient prevention of malignancies or delaying of manifestation to older age by protection against ionizing radiation, control of levels of chromosomal instability and by sufficient intake of antioxidants. Detection of mutation in *NBS1* gene in adult oncology patients plays a less important role in prevention of secondary malignancies than in treated child patients. However, it could be an important preventive measure for their children and other involved relatives and for the improvement of both own clinical prognosis and genetic prognosis of their reproduction.

Abbreviations

AT – ataxia telangiectasia
LOH – loss of heterozygosity
NBS – Nijmegen breakage syndrome
NHL – non-Hodgkin's lymphoma
PCR-SSCP – polymerase chain reaction-single strand conformation polymorphism

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