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PRACA ORYGINALNA

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The presence of *HER2* exon 20 insertion in patients with central nervous system metastases from non-small lung cancer — a potential application in classification for therapy

Obecność insercji w eksonie 20 genu *HER 2* u chorych z przerzutami niedrobnokomórkowego raka płuca do centralnego układu nerwowego — potencjalne zastosowanie w kwalifikacji do terapii

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Abstract

Introduction: HER2 (ErbB2/neu) is a member of the ErbB family of four structurally related receptors of tyrosine kinase activity. Overexpression of ErbB-1 (EGFR) and HER2 is found in many human cancers, but the presence of these genes mutations determines the effectiveness of EGFR and HER2 tyrosine kinase inhibitors in the therapy of non-small cell lung cancer (NSCLC).
Material and methods: To search for insertions of the *HER2* gene in exon 20 in 150 brain metastases of non-small cell lung cancer patients, we used a PCR technique based on analysis of amplified DNA fragment lengths. We also compared the *HER2* mutational status with clinicopathologic features and the presence of *EGFR* and *BRAF* mutations.
Results: *HER2* mutation was present in one male, non-smoking patient with low differentiated adenocarcinoma (0.67% of all patients and 1.5% of patients with adenocarcinoma). The mutations of *EGFR* and *BRAF* genes were not found in HER2-mutated patient.
Conclusions: The literature data suggests that patients with *HER2* mutations may be sensitive to tyrosine kinase inhibitors of both EGFR and HER2 receptors (e.g. afatinib). Therefore, the identification of new driver mutations in NSCLC can improve the quality of patient care by enabling the use of correct molecularly targeted therapies.

Key words: non-small cell lung cancer, brain metastases, HER2 mutation

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Streszczenie

Wstęp: HER2 (ErbB2/neu) jest białkiem należącym do rodziny receptorów HER (EGFR, HER2, HER3 i HER4), posiadających w swej części wewnątrzkomórkowej aktywność kinazy tyrozynowej. Nadekspresja EGFR i HER2 występuje w wielu typach nowotworów, ale to mutacje w genach kodujących te receptory uwrażliwiają chorych na niedrobnokomórkowego raka płuca (NSCLC) na działanie inhibitorów kinaz tyrozynowych EGFR i HER2.

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Materiał i metody: Wykorzystano technikę PCR oraz analizę długości fragmentów amplifikowanego DNA w celu zidentyfikowania u 150 chorych insercji 12 par zasad w obrębie eksonu 20 genu *HER2* w przerzutach NDRP do mózgu.
Wyniki: W guzie z wykrytą mutacją *HER2* nie stwierdzono mutacji *EGFR* ani *BRAF*. Insercja w eksonie 20 genu *HER2* została wykryta u 77-letniego niepalącego mężczyzny chorego na niskozróżnicowanego raka gruczołowego (0,67% wszystkich chorych oraz 1,5% chorych na raka gruczołowego). U tego chorego nie zidentyfikowano innych nieprawidłowości genetycznych.
Wnioski: W literaturze opisano, że u chorych posiadających mutację w genie *HER2* mogą okazać się skuteczne inhibitory specyficzne w stosunku do kinaz tyrozynowych obu receptorów: EGFR i HER2 (np. afatynib). Dlatego też identyfikacja nowych mutacji kierujących w komórkach NSCLC wydaje się kluczem do właściwej kwalifikacji do terapii ukierunkowanych molekularnie.

Słowa kluczowe: niedrobnokomórkowy rak płuca, przerzuty do mózgu, mutacja *HER2*
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Introduction

The family of HER (ErbB) receptors consists of four receptors: HER1 (EGFR, ErbB1), HER2 (Neu, ErbB2), HER3 (ErbB3) and HER4 (ErbB4), which all have in their intracellular part a domain of tyrosine kinase activity. The abbreviation ErbB comes from the name of a viral oncogene (erythroblastic leukaemia viral oncogene) that is connected with the occurrence of erythroblastic leukaemia, and whose structure is homologous with the structure of human HER receptors. The signal for proliferation of epithelial cells is transmitted to a nucleus as a result of homo- or heterodimerization of HER receptors and after stimulation of such complexes by relevant ligands. A preferable partner in heterodimerization for EGFR (epidermal growth factor receptor) is HER2 receptor. Overexpression of HER2 receptor is strongly connected with formation of breast carcinoma. Whereas mutations in tyrosine kinase domain within the EGFR gene-especially deletions in exon 19 and L858R substitution in exon 21 of this gene — that direct bronchial epithelial cells to carcinogenesis occur in approximately 10% of Caucasian patients. Furthermore, the presence of *EGFR* mutation conditions the effectiveness of EGFR tyrosine kinase inhibitor (IKT EGFR). Some researchers also report that *HER2* mutations (to a lesser degree high expression of this receptor) may be the cause for some NSCLC cases [1–5].

Mutations in HER2 tyrosine kinase domain are very rare in NSCLC patients. Preliminary data report that they concern less than 2% of the total population of patients. Usually they are diagnosed in non-smoking females with adenocarcinoma of the lungs. The most important mutations are two different insertions of twelve pairs of nucleotides that disturb the reading frame in exon 20 of *HER2*: A775YVMA (66% of all mutations detected in the *HER2* gene) or M774AYVM. These mutations are analogous with the insertion of nine base pairs in exon 20 of the *EGFR* that makes the structure of HER2 tyrosine kinase domain resemble the structure of EGFR tyrosine kinase domain, which has been modified by the mentioned mutations. Therefore, it is assumed that mutations A775YVMA or M774AYVM of the *HER2* gene result in similar consequences as mutations in exon 20 of the *EGFR* gene. Taking into account that HER2 receptor heterodimerizes in a preferential way with receptor for EGF, the structure of the ATP binding pocket, which is caused by mutation in exon 20 of the EGFR or HER2 gene in heterodimer EGFR/HER2, leads to the growth of tyrosine kinase activity of these receptors. This results in excessive phosphorylation of subsequent signal peptides, proliferation of neoplastic cells, and resistance (or smaller sensitivity) to the action of reversible IKT EGFR [4, 6–11].

The presence of mutations A775YVMA or M774AYVM in the *HER2* gene may, in future, be a potential predictive marker of successful IKT EGFR therapy, and it may also become the goal for new molecularly targeted therapies. The investigation of insertion in exon 20 of the *HER2* may be as crucial for therapy planning as the evaluation of the presence of T790M mutation in the *EGFR* gene, which currently seems to be the main cause for resistance to IKT EGFR (50% of resistance cases). It should be remembered that many patients start IKT EGFR therapy when they are at advanced stage of NSCLC, and brain metastases are the most frequent place of metastases from adenocarcinoma of the lungs. Unfortunately, we know little about the frequency of occurrence of mutations in the *HER2* gene in metastatic tumours from NSCLC and in patients with pathomorphological diagnosis other than adenocarcinoma.

Material and methods

The research included retrospectively 150 patients (102 males and 48 females) from 38 to 81 years of age (59.8 ± 8.8 years), whose paraffin material of neoplastic tissue collected from metastatic foci in the central nervous system from NSCLC was available. The patients underwent standard neurosurgical operation of palliative character.

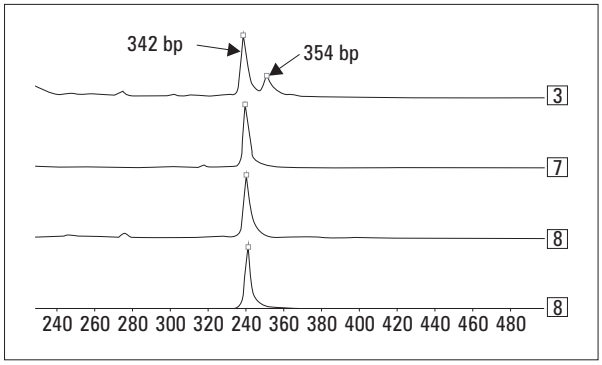

Figure 1. Exemplary analysis of mutation in the *HER2* gene. Double peaks on line no. 3 are evidence for the presence of two PCR products that differ in number of base pairs (bp): the shorter one with 342 base pairs, typical of the wild *HER2* gene, and the longer one (12 base pairs insertion) typical of *HER2* mutant gene. A single peak of the size of 342 base pairs on the lines 7–9 proves solely the presence of a wild -type *HER2* gene. The experiment did not use a positive control presenting the result of DNA amplification with sequence with *HER2* mutation due to lack of commercial availability of DNA with such a sequence

Thirty-two patients had available material from a primary tumour collected during operations, thoracoscopy, endobronchial or transbronchial biopsy, or taken through the chest wall.

Sixty-six patients were diagnosed with adenocarcinoma of the lungs (44%). Squamous cell carcinoma was diagnosed in 24 cases (16%), and giant cell carcinoma in 22 patients (15%); 38 patients (25%) were not diagnosed with any type of NSCLC (not otherwise specified — NOS). The median of the time of the patients' life from the moment of diagnosis until death was 9.2 months. None of the patients was treated with IKT EGFR.

DNA was isolated from paraffin material, including neoplastic tissue of metastatic and primary NSCLC tumours, by using a Qiamp DNA FFPE tissue kit (Qiagen,USA). The evaluation of insertion in exon 20 of the *HER2* was performed by using PCR assay with the help of a pair of primers flanking the mutated region of the *HER2* gene. One of the primers was determined by fluorochrome Cy5. The analysis of the length of amplified DNA fragments was conducted in ALF Express II sequenator by using ALFWin Fragment Analyzer. Additionally, the presence of deletion in exon 19, substitution T790M, substitution L858R in the *EGFR* gene, and mutation V600E in the *BRAF* gene was studied in the investigated material.

Results

The described method appeared to be sensitive enough to evaluate the presence or lack of mutation in the *HER2* gene in all examined patients (Fig. 1). Furthermore, analysis of the occurrence of mutations in the *EGFR* and *BRAF* genes was carried out in the examined group. Mutations in the *EGFR* gene in 9 patients (6.29%) and no mutation in the *BRAF* gene were discovered. However, the presence of an insertion in exon 20 of the *HER2* (insertion variant A775YVMA or M774AYVM) was found only in one patient who had available material from a metastatic tumour from NSCLC (0.67% of all examined patients and 1.5% of patients with diagnosis of adenocarcinoma). This mutation was found in a 77-year-old non-smoking male, who had been diagnosed with advanced low differentiated adenocarcinoma of the lungs with cerebellar metastases. Due to his poor condition after neurosurgical operation, and the progression of the disease, the patient was not treated with chemotherapy, radiotherapy or other molecularly targeted therapy. Additional molecular examinations of the patient excluded the presence of activating mutations in exon 19, exon 20 (T790M) and exon 21 (L858R) of the *EGFR* gene or substitution V600E in the *BRAF* gene.

Discussion

The present study proves that primary mutations in the *HER2* gene may be discovered in Polish patients with NSCLC, not treated with molecularly targeted therapies. Furthermore, mutation in the *HER2* gene has been noted in metastatic cerebellum tumours from NSCLC, which is the first such report in the world. Mutations in the *HER2* gene are probably responsible for the development of the forms of lung cancer that do not depend on tobacco smoke carcinogens, especially adenocarcinoma. However, the occurrence of mutations in the *HER2* gene in Caucasian patients is very rare. This research found it in less than 1% of NSCLC patients.

Shigematsu et al. searched for mutations in the *HER2* gene in 671 primary NSCLC tumours and in 80 NSCLC cell lines and other types of neoplasms (140). The authors found different types of insertion in exon 20 of the *HER2* gene in 11 patients with NSCLC (1.6%) and in one cell line — adenocarcinoma of the lungs (NCI-H1781). Such mutation has not been discovered in other types of neoplasms, including 55 small cell lung cancers. Mutations in the *HER2* gene were more frequent in non-smokers (3.2%, 8 out of 248 patients) and have occurred exclusively in patients with adenocarcinoma (2.8%, 11 out of 394 patients). Moreover, they were more often in females (2.7%, 7 out of 258 females) than in males (1%, 4 out of 413 males). Only one mutation was diagnosed in

Caucasian patients (0.73%, 1 out of 137 patients) [6]. In another study, Sasaki et al. found only one non-smoking patient with adenocarcinoma who had mutation in the form of insertion of 12 nucleotides in exon 20 of the *HER2* gene among the 95 examined patients with NSCLC [7]. Buttitta et al. diagnosed mutation in the *HER2* gene in 9 of 403 Caucasian patients with adenocarcinoma (2.2%), while 7 mutations were 12-nucleotide insertion within exon 20. Mutations have been found more often in females (4.1%), non-smokers (3.1%) and in patients with pattern of bronchiolalveolar carcinoma (6.2%), although they have been also found in males (1.8%) and in smokers (1.9%) [9].

The results of the quoted research projects and the present study are contradictory to the results obtained by Stephens et al., who declared that mutations in the tyrosine kinase domain in the *HER2* gene (various types of mutations and abnormalities in the *HER2* gene) occur in 4% of NSCLC patients (120 investigated primary tumours), including 10% of patients with adenocarcinoma [9]. Moreover, Lee et al. described 12 carriers of mutations in the *HER2* gene in a group of 202 non-smoking Caucasian patients (6%) who underwent surgery due to adenocarcinoma of the lungs [11].

If we assume that mutations in the *HER2* gene are driver mutations that direct bronchial epithelial cells to carcinogenesis, and that they do not coexist with other driver mutations (coexistence of insertion in exon 20 of the *HER2* gene with some mutations in the *EGFR* and *BRAF* genes has been excluded in the present paper), molecularly targeted therapy directed against the HER2 tyrosine kinase may be sought for patients with this mutation. Tyrosine kinase inhibitors that inhibit exclusively EGFR tyrosine kinase activity (gefitinib and erlotinib) in the case of mutation in the *HER2* gene are ineffective. Lapatinib — double, reversible EGFR and HER2 tyrosine kinase inhibitor has showed activity towards cell lines but it was ineffective in patients with non-small cell lung cancer [12, 13]. Whereas afatinib (BIBW 2992) — irreversible EGFR, HER2 and HER4 tyrosine kinase inhibitor has appeared to be successful in eliminating neoplastic cells with *HER2* mutation in both: cell cultures and animal models [14–18]. Moreover, there are first reports on cases concerning the effectiveness of this drug in females with adenocarcinoma, who were carriers of *HER2* mutation [19]. Experiments *in vitro* on the possible use of therapy combining afatinib and sirolimus (mTOR inhibitor) in patients with *HER2* mutation are being conducted [4]. Additionally, trastuzumab — monoclonal antibody to HER2 extracellular domain, which is successfully used in therapy for women with breast cancer and HER2 overexpression, may be effective in patients with NSCLC from *HER2* mutation [20].

The above discussion shows that analysis of patients' genetic profile may increase the range of possibilities for the use of molecularly targeted therapies in NSCLC patients. It is possible that in the future personalized NSCLC therapy based on examination of many various mutations in neoplastic cells will be the reality.

Conflict of interest

The authors declare no conflict of interest.

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