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Clinical genetics of lung cancer

Genetyka kliniczna raka płuc

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Summary

Every year more than 1 million people all over the world die because of lung cancer. In addition to smoking and other environmental carcinogens, factors responsible for the disease include constitutional DNA alterations. A characteristic feature of markers of a genetic predisposition to lung cancer, is relatively slightly increased risk of developing cancer among carriers of DNA alteration. Very promisive markers of severe risk of lung cancer are biochemical markers like the concentration of certain micro elements. Studies performed in our Center clearly indicate that selenium levels > 80 μg/l are associated with strongly reduced risk of lung cancer. The examination of selenium concentration in blood serum can be very useful marker in screening for early detection of lung cancer by preselection of individuals with high probability of finding cancer by computerized tomography (CT). Very important is also identification of additional predictive markers for adjustment of treatment regimens depending on the status of specific markers, such as the presence or absence of specific mutations.

Streszczenie

Każdego roku na świecie na raka płuc umiera ponad 1 milion ludzi. Poza paleniem tytoniu i innymi środowiskowymi czynnikami rakotwórczymi, wśród czynników odpowiedzialnych za wystąpienie choroby wymienia się zmiany DNA. Cechą charakterystyczną jak dotąd zidentyfikowanych markerów genetycznej predyspozycji do raka płuc jest stosunkowo nieznacznie podwyższone ryzyko zachorowania na nowotwór złośliwy tego narządu w przypadku nosicielstwa zmian DNA. Duże nadzieje w identyfikacji markerów silnego ryzyka raka płuc budzą markery biochemiczne, jak np. stężenie określonych mikroelementów. Badania przeprowadzone w naszym Ośrodku wyraźnie wskazują, że poziomy selenu w organizmie > 80 μg/l związane są z redukcją ryzyka zachorowań na raka płuc. Badanie poziomu selenu w surowicy krwi może być niezwykle użytecznym markerem w badaniach przesiewowych wczesnego wykrywania raka płuc do wstępnej preselekcji osób, u których zasadne jest wykonanie badania kontrolnego z wykorzystaniem tomografii komputerowej (CT). Bardzo ważne jest też poszukiwanie kolejnych markerów predykcyjnych w celu opracowania algorytmów postępowania charakteryzujących tzw. medycynę spersonalizowaną, gdzie schematy leczenia są dostosowane do statusu określonych markerów, np. obecności lub braku określonych mutacji.

INTRODUCTION

Every year in Poland a more than 15 thousands of lung cancer in males and about 5 thousands in women, are recorded, which is respectively, 24 and 8% of all cancer cases. More than 16 thousands Polish men and more than 5 thousands Polish women die each year in Poland due to cancer of this organ (1). Registry of the International Agency for Research on Cancer (IARC) shows that taking into consideration the whole world, each year more than 1 million people: 1 376 579, including 948 993 men and 427 586 women die because of lung cancer (2). In addition to smoking, factors responsible for the occurrence of the disease include exposures on several occupational and environmental carcinogens such as asbestos, arsenic, radon and aromatic hydrocarbons (3).

IDENTIFICATION OF RISK GROUPS

DNA markers associated with increased predisposition to lung cancer

Despite large contribution of lung cancer in the whole incidence of malignant neoplasms, search for markers of predisposition to lung cancer is still a problem to solve. As far, successfully proven as markers of high genetic predisposition to breast cancer, the genetic basis for risk of lung cancer is relatively poorly understood and genetic markers of high risk for lung cancer are not identified. The following changes in DNA are described for Caucasians as related with increased genetic susceptibility to lung cancer (own elaboration on the basis http://www.megabionet.org/bio/hlung/lung_cancer/SNP_list.jsp) (tab. 1).

It can be summarized, that generally markers of genetic predisposition to lung cancer are slightly increasing risk of tumors among carriers of DNA changes. In addition to the changes associated with an increased risk of lung cancer it has been shown that a change Ile157Thr (rs17879961) in the gene *CHEK2* is associated with a reduced risk of lung cancer (OR – 0.3, $p = 3 \times 10^{-8}$) (4). This association was first demonstrated by researchers at our center, and recently it has been confirmed in a second independent multicenter study (OR – 0.38, $p = 1.27 \times 10^{-13}$) (5).

Selenium as a marker of high risk

Recent research indicates that the selected micro-, macronutrients and vitamins may also significantly affect the risk of development and progression of malignancies. It is known that certain amounts of vitamins and micro- and macronutrients are essential to the proper functioning of the body. Micro- and macronutrients can be incorporated into the active site of enzymes and warrants their activity in a variety of biological processes. Both their deficiency or excess can cause dysfunctions leading to major diseases, including cancer. One of these micronutrient is selenium. Studies conducted in our center clearly show that the levels of selenium in the serum > 80 μg/l are associated with reduced risk of lung cancer. In comparison with a group of people, in whom the level of selenium in the serum reached the value < 60 μg/l, the difference is approx. 10 times (OR – 0.10; 95% CI 0.03-0.34; p = 0.0002) (6). This observation is very important in the context of rational planning and implementation of screening in individuals at increased risk of developing lung cancer. The study of serum selenium levels in peripheral blood can be extremely useful marker for initial pre-selection of people for whom it is reasonable to perform control study using computerized tomography (CT). Scheme of such screening should first include analyses of the level of selenium in people who are at increased risk of lung cancer (people of both sexes aged > 55 years and having a history of smoking in the amount of > 20 pack-years) and then CT in patients with the lowest levels of selenium in the blood serum. Results of American studies indicate that low-dose CT is characterized by much greater sensitivity of detection of early stages of lung cancer compared to conventional radiography. The application of this diagnostic technique in risk groups with a frequency of once every two years increases the number of diagnosed early stages of lung cancer and consequently contributes to a reduction in mortality from this cancer (7-9). It seems that the combination of this observation together with the measurement of the concentration of selenium in the blood serum of patients has a real prospect for more medically and cost-effective prevention of cancer. Such actions we performed in cooperation with the Regional Hospital of Lung Diseases in Szczecin-Zdunowo.

Prognostic molecular markers

Another practical aspect of the use of the markers is their prognostic usefulness. The use of markers to evaluate the prognosis for survival, the possibility of metastasis or recurrence are the most important prognostic markers application. Studies have shown that the occurrence of tumor cells with KRAS gene mutation is associated with shorter survival in patients with small cell lung cancer (Non-Small Cell Lung Cancer – NSCLC) (10). Strong predictive value of KRAS somatic mutations was also demonstrated in relation to the increased risk of relapse and shorter survival, regardless of clinical stage and histological type of NSCLC (11). Independently on KRAS mutations occurrence of somatic mutations of the p53 gene is a negative prognostic factor for patients with adenocarcinoma. This relationship is not found for patients with squamous cell carcinoma (12). Total concentration of circulating DNA in peripheral blood and micro RNA are also of prognostic value. Increased growth of DNA concentration in patients with NSCLC after surgery was observed in recurrences or incomplete resections. The DNA concentration in plasma after radical resection of the tumor was more than 3-fold lower than for a failure or relapse treatment (13-15). Also promoter hypermethylation of CDKN2A/p16 and FHIT was associated with shorter progression-free and a higher risk of recurrence in patients operated of stage I NSCLC (16). However, a positive predictor is expression of p21 and p16 in the resectable material of patients operated of stage N2 NSCLC (17).

Predictive molecular markers

Predictive molecular markers are used in clinical therapy of lung cancer. Thus, for example *BRCA1* gene overexpression in lung cancer is associated with increased susceptibility to the effects of vinorelbine and other drugs that bind tubulin. Whereas methylation of *BRCA1* (Breast Cancer 1, early onset 1) is associated with the increased susceptibility to cisplatin and its derivatives and the reduction of response to antimitotically active formulations (18). High expression of genes *ERCC1* (Excision Repair Cross-complementing rodent rep air deficiency, Complementation

Table 1. Changes in genes associated with increased risk of lung cancer.

Gene symbol	Change	Gene name	SNP	OR	p
TNC	A1781T	Tenascin C	rs2274750	1.32	0.025
CAMKK1	E375G	Calcium/calmodulin-dependent protein kinase kinase 1, alpha	rs7214723	1.37	$5.4x10^{-5}$
AKAP9	M463I	A kinase (PRKA) anchor protein (yotiao) 9	rs6964587	1.32	$1.0x10^{-4}$
AKAP10	R249H	A kinase (PRKA) anchor protein 10	rs2108978	1.25	0.0085
GHR	P495T	Growth hormone receptor	rs6183	12.98	0.0019
GHRH	L75F	Growth hormone releasing hormone	rs4988492	1.44	0.0453
BRCA2	K3326X	Breast cancer 2, early onset	rs11571833	1.72	0.0075
XRCC4	I137T	X-ray repair complementing defective repair in Chinese hamster cells 4	rs28360135	1.31	0.0205
IGFBP5	R138W	Insulin-like growth factor binding protein 5	rs11575194	1.29	0.027
BAT4	R41L	HLA-B associated transcript 4	rs3130618	1.26	0.0005
NRIP1	R448G	Nuclear receptor interacting protein 1	rs2229742	1.24	0.0052
HIF1AN	P41A	Hypoxia-inducible factor 1, alpha subunit inhibitor	rs2295778	1.34	0.007
CDH12	V68M	Cadherin 12, type 2 (N-cadherin 2)	rs4371716	1.63	0.0001
ZNF24	N220S	Zinc finger protein 24	rs2032729	1.24	0.0124
MSH4	S914N	MutS homolog 4 (E. coli)	rs5745549	1.27	0.0461
CFTR	R75Q	Cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7)	rs1800076	1.27	0.0412
PLCD1	R257H	Phospholipase C, delta 1	rs933135	1.45	0.0312
BARD1	R658C	BRCA1 associated RING domain 1	rs3738888	1.59	0.0329
GPR68	R63Q	G protein-coupled receptor 68	rs2230339	6.80	0.0213
FASN	R1694H	Fatty acid synthase	rs2229424	6.79	0.0214
LOC123688	C/T intron	–	rs8034191	1.32	$9x10^{-10}$
CHRNA3	215 Y/Y	Acetylcholine receptor subunit 3	rs1051730	1.30	$5x10^{-9}$
CHRNA3	C/T Intron	Acetylcholine receptor subunit 3	rs12914385	1.29	$4.79x10^{-16}$
CHRNA5	D398N	Acetylcholine receptor subunit 5	rs16969968	1.32	$3x10^{-9}$
TERT/CLPTM1L	C/T intron	Human telomerase reverse transcriptase	rs402710	1.21	$7x10^{-5}$
CLPTM1L (CRR9)/TERT	A/C intron	Cleft lip and palate transmembrane 1 like	rs2736100	1.15	0.016
TERT	305 A/A	Human telomerase reverse transcriptase	rs2736098	1.21	0.016
BAT3	G/T intron 1	HLA-B associated transcript 3	rs3117582	1.20	$4.9x10^{-9}$
RAD52	C/T	Yeast, homolog of *RAD52*	rs6489769	1.20	2.3×10^{-8}
MSH5	G/A	MutS homolog 5 (E. coli)	rs3131379	1.52	0.001
MHC complex	A/G	Major histocompatibility complex	rs4324798	1.28	$4x10^{-7}$
TNXB	T/C , T302A	Tenascin XB isoform 1	rs1150752	1.24	$1.93x10^{-6}$
CYP1B1	432 V/L	Cytochrome P450 1B1	rs1056836	1.97	0.001
ERCC5	A/C Intron	Excision repair cross-complementing rodent repair deficiency, complementation group 5 (xeroderma pigmentosum, complementation group G (Cockayne syndrome))	rs732321	2.39	<0.038
MLH3	C/T Intron	MutL homolog 3 (E. coli)	rs175057	1.52	<0.044
MLH3	G/A 844 P/L	MutL homolog 3 (E. coli)	rs175080	1.60	<0.039
POLD3	T/G	Polymerase (DNA-directed), delta 3, accessory subunit	rs10857	1.84	<0.001
POLD3	C/A Intron	Polymerase (DNA-directed), delta 3, accessory subunit	rs41541119	2.66	<0.03
RECQL4	C/T, 44E/E	RecQ protein-like 4	rs2306386	1.35	<0.034
RECQL4	C/T	RecQ protein-like 4	ss70347238	1.35	0.034
MMP1	1831 C/T Intron	Matrix metallopeptidase 1 (interstitial collagenase)	rs996999	1.3	0.0152
MMP1	12471 G/A, Intron	Matrix metallopeptidase 1 (interstitial collagenase)	rs193008	1.5	0.04
MMP1	7229 C/T Intron	Matrix metallopeptidase 1 (interstitial collagenase)	rs1938901	1.5	0.0534
FANCA	C/T	Fanconi anemia, complementation group A	rs7204478	1.66	0.004
RAD52	A/C Intron	RAD52 homolog isoform alpha	rs3748521	1.83	0.029
DNA-PK PRKDC	C/G Intron	Protein kinase, DNA-activated, catalytic	rs1231204	2.20	0.041
LIG1	-7 C>T	Ligase I, DNA, ATP-dependent	rs20579	1.73	<0.01
LIG3	IVS9-21 A>G	Ligase III, DNA, ATP-dependent	rs3730931	1.73	0.01
CARD15/NOD2	3020insC	NOD2 protein	rs5743293	1.7	0.03
CDKN2A	G/A, A148T	Cyclin-dependent kinase inhibitor 2A	rs3731249	2.0	0.0052
MSH5	G/A intron	MutS homolog 5 isoform c	rs3131379	1.40	0.037
6q24.2 – STX11	T/C	Syntaxin 11	rs4286803	1.25	0.012
7p21.2 – ETV1	C/T	Ets variant gene 1	rs984468	1.20	0.033
12q24 – ALDH2	A/G intron	Aldehyde dehydrogenase 2	rs4767364	1.12	$2x10^{-7}$
4q21 – HEL308, FAM175A	C/T I306V	DNA helicase HEL308	rs1494961	1.12	$4x10^{-8}$
RPA1	A/G Intron	Replication protein A1, 70kDa	rs2287321	1.87	0.012
MSH3	A/G Intron	MutS homolog 3	rs6151838	1.65	0.026
PMS2	A/C Intron	PMS2 postmeiotic segregation increased 2 isoform	rs2286681	1.42	0.021
POLR2C	A/G Promotor	DNA directed RNA polymerase II polypeptide C	rs686402	1.47	0.039
FANCC	T/C Intron	Fanconi anemia, complementation group C	rs3737142	1.31	0.025
FANCC	C/T Intron	Fanconi anemia, complementation group C	rs3780564	1.29	0.017
RAD51	A/G Intron	RAD51 homolog protein isoform 1	rs2304579	1.72	0.037
DNA-PK	C/G Intron	Protein kinase, DNA-activated, catalytic	rs1231204	2.86	0.036
XRCC4	G/A Intron	X-ray repair cross complementing protein 4	rs177297	1.53	0.042
XRCC4	T/C Intron	X-ray repair cross complementing protein 4	rs307316	1.86	0.004
XRCC3	722 C/T Thr/Met	X-ray repair cross complementing protein 3	rs861539	1.96	0.02
RAD23B	C/T Ala249Val	UV excision repair protein RAD23 homolog B	rs1805329	1.3	–
ERCC4	G/A Arg415Gln	Excision repair cross-complementing rodent	rs1800067	1.5	–
ERCC5	G/C Asp1104His	XPG-complementing protein	rs17655	1.4	–
CCND1	G/A	Cyclin D1	rs9344	2.52	0.013
CYP1A1	A/G I/Val	Cytochrome P450, family 1, subfamily A	rs1048943	2.3	–
ADH1C	C/T, Arg272Gln	Class I alcohol dehydrogenase, gamma subunit	rs1693482	1.24	-

group 1) and *RRM1* (Ribonucleoside-diphosphate Reductase M1) is associated with shorter survival. The low level of expression correlates with longer survival of patients with advanced NSCLC treated with cisplatin and gemcitabine (19). In other words, a low level of expression of *ERCC1* and *RRM1* gene is a preferred predictor of response to treatment with such compounds like cisplatin and gemcitabine, and simultaneously a negative prognostic factor for patients not exposed to such treatment (20). The most important and perhaps the only recognized for clinical value are somatic mutations in the *EGF* gene. Their presence is associated with advantageous response to treatment with tyrosine kinase inhibitors (Tyrosine Kinase Inhibitors – TKI), for example erlotinib, gefitinib in patients with NSCLC (21). However, it should be noted, that these mutations occur only in 10% of patients with NSCLC Caucasian although in 30-67% of patients of Asian origin (22). Meta-analysis of 7 phase II studies clearly showed, that the use of the kinase domain mutations of the *EGFR* gene as the main inclusion criteria to TKI treatment resulted in an increase in the percentage of positive responses to treatment, to 87% and prolonged progression-free survival time from 7.7 to 14 months. In addition, a favorable response to treatment was independent of gender, race, and smoking (23). The study showed also, that patients without mutations activating EGFR in the primary tumor achieve greater benefit from the application of classical chemotherapy than TKI therapy (24). Implementation in clinical practice of further reliable predictive markers is essential for the effective treatment of lung cancer.

CONCLUSIONS

The absence of strong genetic predisposition markers for lung cancer implicates the search for other markers, the use of which could improve the efficiency/effectiveness of screening. High hopes are biochemical markers, such as concentrations of specific micronutrients. The first of such markers, is the concentration of selenium in blood serum. Further work is ongoing on new markers from a group of micronutrients. It is important also to search for further predictive markers in order to develop algorithms of personalized medicine, where treatment regimens are adjusted to the status of the markers, for example the presence or absence of specific mutations.

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