HSPA2 Is Expressed in Human Tumors and Correlates with Clinical Features in Non-small Cell Lung Carcinoma Patients

DOROTA SCIEGLINSKA[1], AGNIESZKA GOGLER-PIGLOWSKA[1], DOROTA BUTKIEWICZ[1], MYKOLA CHEKAN[2], EWA MALUSECKA[1], JOLANTA HARASIM[3], ANNA HABRYKA[1] and ZDZISLAW KRAWCZYK[1]

[1]*Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland;*
[2]*Department of Tumor Pathology, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland;*
[3]*Department of Thoracic Surgery, The Medical University of Silesia, Zabrze, Poland*

Abstract. *Background/Aim: It has been shown that HSPA2 protein, a testis-enriched member of HSPA/HSP70 family, is important for cancer cell growth and metastasis. However, the status of HSPA2 expression in tumors and its clinical/prognostic significance are obscure. Herein we aimed to investigate the expression of HSPA2 in various types of tumors and to determine the possible clinical and prognostic significance of HSPA2 in non-small cell lung carcinoma (NSCLC). Materials and Methods: Tissue microarrays and postoperative NSCLC tumors were tested for HSPA2 by immunohistochemistry. Results: HSPA2 is expressed in the majority of tumor histotypes. In NSCLC patients (n=85), nuclear HSPA2 expression was associated with histology, TNM staging and prognosis. High HSPA2 expression was significantly related to shorter overall survival (OS) in stage I-II patients. In multivariate analysis, high HSPA2, together with stage IIIA and male sex, were associated with shorter OS in the whole group. Conclusions: As exemplified in NSCLC the status of HSPA2 in human tumors may have certain prognostic significance.*

The human *HSPA2* gene, a poorly-characterized member of the HSPA (*HSP70*) multi-gene family, codes for heat shock proteins (HSP) of 70 kDa molecular weight. In humans, the HSPA family consists of at least thirteen highly homologous genes, which are either constitutively expressed and/or their expression can be induced in response to various pathological conditions and environmental stress (1, 2). Some of HSPA proteins are well-known cytoprotective agents, their activity is beneficial for survival of normal and pathologically-transformed cells exposed to stress condition (1-3).

Originally, *HSPA2* was identified in rodents as a testis-specific gene essential for development of male germ cells (4-6). In mouse spermatocytes HSPA2 was found to be involved in synaptonemal complexes disassembly and formation of active CDC2/cyclin B1 complex (6-8). In contrast to its rodent orthologs, the human *HSPA2* gene is highly transcribed in multiple somatic tissues (9, 10). Recently, using a highly specific anti-HSPA2 antibody, we have shown that HSPA2 is expressed in a cell-type specific manner in human somatic tissues (11). The functions of HSPA2 in somatic cells are elusive. Recently, its general chaperoning activity (re-folding of denatured luciferase) has been presented (12). We have also shown that HSPA2 can be part of the cytoprotective system, its overexpression improves the ability of cells to cope with proteotoxic stress induced by heat shock and proteasome inhibitors (13).

HSPA2 can also be expressed in human cancer cell lines (14-17) and in some primary tumors (16-18). Published evidence, although very limited, suggests that HSPA2 is important for cancer cell biology. *HSPA2* gene silencing reduces proliferation and induces senescence of cervical and breast cancer cell lines (14), as well as affecting the motility and invasiveness of urothelial and cervical cancer cell lines (17, 18). Therefore, a critical role for HSPA2 in growth and metastatic potential in cancer cells has been assumed. Concurrently, the above data form a strong incentive to investigate whether HSPA2 could be considered as a clinically-relevant marker. The potential use of HSPA2 as a tumor marker should be assessed taking into account the knowledge on its expression in a neoplastic and corresponding normal tissue. Data on HSPA2 expression in

Correspondence to: Dorota Scieglinska, Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland. Tel: +48 322789679, Fax: +48 322313512, e-mail: dorotas@io.gliwice.pl

Key Words: Heat shock proteins, HSPA2, immunohistochemistry, NSCLC, prognosis.

selected normal tissues has just been published (11), but little is known about HSPA2 in primary tumors (17, 18).

In the work presented herein, we investigated by immunohistochemistry (IHC) the HSPA2 expression in various types of human tumors using tissue microarrays (TMAs) and polyclonal HSPA2 antibody of proven specificity, which discriminates between HSPA2 and other highly similar HSPA proteins (11, 16). The analysis of HSPA2 expression in post-operative specimens from 85 non-small cell lung carcinoma (NSCLC) patients revealed the association of HSPA2 expression with histological type of tumor, TNM staging and overall survival (OS).

Materials and Methods

Tissues. Tissue microarrays (TMA) slides with catalog number #LC242 and #MC501 were purchased from Tissue Array Network (Rockville, USA). These TMAs contain formalin-fixed, paraffin-embedded samples of human tumors and normal (or cancer adjacent) tissues. The TMA #LC242 (1.5 mm core diameter) contains 11 cases of lung NSCLC. The TMA #MC501 (0.6 mm core diameter) contains most common types of cancer (from 8 to 19 cases/type) and normal controls (5 cases/type, not included for each cancer type). Details are presented in Table I.

We tested archival material of surgically-resected tumors from 85 NSCLC patients and 5 cases of adjacent non-tumor lung tissues (paired). The protocol of the study was approved by the Ethics review board (no KB/430-66/12; 14-Nov-2012). All patients were operated on between 1993-1995 at the Department of Thoracic Surgery of the Silesian Medical University in Zabrze (headed by K. Czyzewski). None of the patients received chemo- or radiotherapy prior to surgery. Clinical staging was assessed according to the 1997 AJCC classification. The majority of patients were tobacco smokers. Table II shows details on clinicopathological characteristics of the patients. Median follow-up was 111.4 ranging from 76.6 to 146.2 months and 62 (76.5%) deaths occurred during the observation period. Survival information was available for 81 of 85 patients, four patients were lost from follow-up.

Immunohistochemistry (IHC). For HSPA2 detection, a rabbit mono-specific polyclonal antibody of proven specificity was used (11, 16). Briefly, anti-HSPA2 serum was generated in rabbit by injection of C-terminal peptide from mouse HSPA2 protein (NH2-SKLYQGGPGGGGSSGGPT; amino acids 611-628), crude anti-serum was antigen affinity purified, as described in (11, 16). The IHC procedure was performed as described previously using an IMPRESS kit obtained from Vector Laboratories (Janki, Poland) (11). Briefly, prior to IHC citrate-mediated (0.01M buffer pH 6.0) high-temperature antigen retrieval was performed (19). The IHC controls include: (1) omission of the primary antibody; (2) peptide competition (Figures 1 and 2). For peptide competition, the antibody in working concentration was preincubated (1 h, RT) with mass excess of the peptide antigen (1 μg).

The HSPA2 expression was semi-quantitatively assessed by assigning a proportion score and an intensity score. The proportion score was assigned according to proportion of tumor cells with positive nuclear/cytoplasmic staining (0, <1%; 1, 1% to ≤10%; 2, 10% to ≤30%; 3, 30% to ≤60%; 4, >60%). The intensity score was assigned for the average intensity of positive tumor cells (0, none; 1, weak; 2, moderate; 3, strong). The intensity and proportion scores were multiplied and, finally, strong staining was assigned to 12-8 scores, moderate to 6-4, weak to 3-1, negative to 0. All specimens were analyzed by three independent investigators (D.S., A.G-P. and M.C.).

Statistical analysis. The Pearson's Chi-square test was used to compare all categorical variables. OS was defined as the time from diagnosis until death from any cause or last known date alive. The Kaplan-Meier survival curves were compared with the log-rank test. The Cox proportional hazards regression model was used to estimate crude or adjusted hazard ratios (HRs) and 95% confidence intervals (CIs). For survival data analysis, the HSPA2 expression was dichotomized as low (negative and weak staining) or high (moderate and strong staining). Adjustment for age, sex, histology (SCC *versus* AC), clinical stage (I-II *versus* IIIA) and smoking pack-years was applied. Age and number of pack-years were examined as continuous variables, while all others were categorized. The level of significance was set at 0.05 and calculations were performed using STATISTICA v8.0 (StatSoft Inc., Tulsa, OK, USA).

Results

Tissue microarray study. The IHC screening of HSPA2 expression was performed on 268 samples representing 14 types of primary human tumors (8 to 19 individual cases for each type). In total, HSPA2-positive cancer cells were found in 26% of the cases and in 11 out of 14 analyzed types of tumors (Table I). Staining was detected in the nuclei and/or the cytoplasm of cancer cells. The highest numbers of HSPA2-positive cases were found in skin SCC (9/9 – number of cases/number of HSPA2-positive cases), breast infiltrating lobular (ILC; 10/7), breast non-specific infiltrating ductal (IDC; 17/10), head and neck SCC (HNSCC; 16/8), and ovary papillary serous (PSC; 12/6) cancers. Serious limitations of our study is the small size of arrayed samples. The main concern is that small cores may not represent the whole tumor due to intra-tumoral heterogeneity, that would possibly cause false-negative results. Therefore we do not discuss the tumor types which appeared to be HSPA2-negative (Table I).

Previously, it has been shown that HSPA2 is expressed in a cell- and tissue-type specific pattern in human tissues (11). We collated the published data on the HSPA2 expression in normal tissues (11) with data on its expression in tumors presented here. Such a comparison enabled identification of pairs of tumors and corresponding normal tissues for which HSPA2 was merely found in tumors (Figure 1). This phenomenon could pertain to breast non-specific IDC and ILC (Figure 1a), bladder TCC (Figure 1b), pancreatic AC and liver AC (Figure 1d). However, interestingly in some cases HSPA2 protein was found in both the tumors and corresponding normal tissue. For example, HSPA2 was detected in all specimens of skin SCCs (Table I) and in normal epidermis (11), specifically in the basal layer cells (Figure 1c) (Table I).

Table I. *HSPA2 expression in various histological types of human tumors – results of TMA-based IHC analysis.*

Organ	HSPA2 in normal tissue[a]	Pathology diagnosis	No/HSPA2-positive[b]	HSPA2 immunostaining[c]		
				Strong (n)	Moderate (n)	Weak (n)
Skin	Epidermis – keratinocytes of basal layer	SCC	9/9	4	1	4
		MM	19/7	3	1	3
Breast	0	Non-specific IDC	17/10	5	0	5
		ILC	10/7	1	2	3
Lung	Bronchial epithelium – goblet cells, basal cells, ciliated cells	SCC	17/5	1	1	3
		AC	8/0	-	-	-
Ovary	-	PSC	12/6	0	1	3
Testis	Spermatocytes and spermatids	Seminoma	15/2	0		1
Kidney	Kidney cortex – distal tubules	Clear-cell RCC	8/2	-	-	2
		Granular-cell RCC	10/0	-	-	-
Thyroid	0	PC	10/0	-	-	-
		FC	9/0	-	-	-
H&N	-	SCC	16/8	2	2	4
Bladder	0	TCC	19/4	1	1	2
Liver	0	HCC	18/3	-	-	3
Pancreas	0	AC	18/3	-	-	3
Stomach	Epithelium – parietal cells	AC	18/2	-	-	2
Prostate	0	AC	16/1	-	-	1
Colon	Epithelium – goblet cells; smooth muscle fibers of lamina muscularis mucosae	AC	17/8	2	1	5

[a]Refers to Figure 1 and Reference (10). 0: HSPA2 protein not detected; –: no data available. SCC: Squamous cell carcinoma; MM: malignant melanoma; IDC: infiltrating ductal carcinoma; ILC: infiltrating lobular carcinoma; AC: adenocarcinoma; PSC: papillary serous carcinoma; RCC: renal cell carcinoma; PC: papillary carcinoma; FC: follicular carcinoma; TCC: transitional cell carcinoma; HCC: hepatocellular carcinoma; [b]HSPA2-positive refers to tumors showing HSPA2 staining in nucleus, nucleus/cytoplasm and cytoplasm. [c]scoring of HSPA2 immunostaining based on nuclear staining. n: Number of cases.

Association of HSPA2 expression with clinical data and survival of resected NSCLC patients. In the second part of our study, we analyzed HSPA2 expression in whole sections of resected NSCLC tissues (n=85) and in corresponding cancer-adjacent normal lung tissues (n=5). We focused on NSCLC because in our previous report we found HSPA2 expression at the highest level in cell lines derived from this tumor type (16). In normal lung tissues, HSPA2 immunostaining was observed in bronchial epithelium, in nuclei of basal cells and in apical cytoplasm of ciliated cells. No staining was found in ciliated simple columnar epithelium and pulmonary alveoli (Figure 2a).

The NSCLC group comprised of 56 SCC and 29 AC cases (Table II). Nuclear staining was found in 53 cases (62%), but in 18 (21%) of them HSPA2 immunoreaction was also visible in the cytoplasm (Table III) (Figure 2). The separate scoring of nuclear and cytoplasmic HSPA2 staining revealed that in each case the nuclear score was higher or equal to the cytoplasmic one. Accordingly, it seems that scoring of HSPA2 expression can be based on independent nuclear staining alone.

IHC analysis showed a high heterogeneity of HSPA2 expression (Figure 2). HSPA2-stained cells were distributed preferentially at the periphery of tumor cell clusters (Figure 2). In fact, the staining was homogenous only in a subset of cases scored as strong (Figure 2b), and 65% of positive cases showed staining in less than 60% of cancer cells (Figure 2c and d). Less than 10% of cancer cells were stained and the majority of cases (14 out of 18) scored as weak (Figure 2d).

The correlation of clinicopathological and survival data of NSCLC patients with IHC scores was performed separately for nuclear and cytoplasmic staining. Nuclear HSPA2 staining showed an association with histological type of tumor, TNM staging and patients' survival. For the whole group, the HSPA2-positive cases were more frequent in SCC than in AC (73% *vs*. 41%, p=0.008), the majority (93%) of strongly-stained cases were SCCs. In SCC patients strong and moderate staining were more frequent in the early stage (IB + IIB) than in advanced stage (IIIA) subgroup (66% *vs*. 24%, p=0.006). No statistically significant associations between IHC data and smoking status, as well as TNM staging for AC cases were detected.

In order to assess a potential prognostic value of HSPA2, we performed survival analysis for 81 patients for whom relevant data were available (Table IV). In this group, the median OS was 16.8 (95%CI. 6.2-27.4) months. For

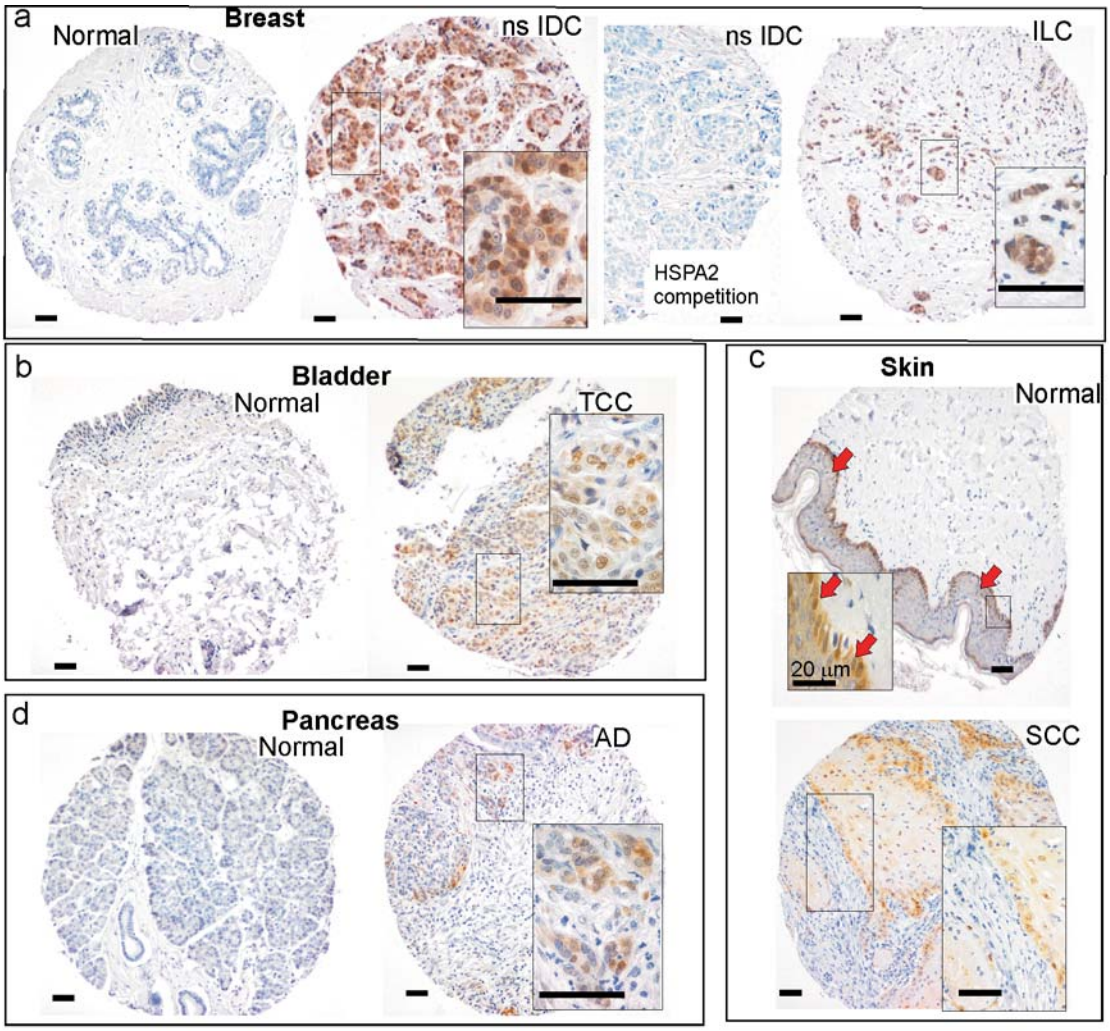

Figure 1. *Examples of HSPA2 detection in various human tumors. TMAs analysis. a-d: Examples of HSPA2 staining in specimens of breast (a, strong), bladder (b, moderate), skin (c, strong) and pancreas (d, weak) cancer. HSPA2 was detected in the nuclei and cytoplasm of cancer cells. No HSPA2 was found in normal breast (a, right), bladder (b, left) and pancreas (d, left). HSPA2-positive keratinocytes (red arrows) are visible in basal layer of normal epidermis (c, top). HSPA2 competition: negative control showing results of peptide competition reaction. ns IDC: Non-specific infiltrating ductal carcinoma; ILC: infiltrating luminal carcinoma; TCC: transitional cell carcinoma; SCC: squamous cell carcinoma; AD: adenocarcinoma; Bar: 100 μm.*

analysis, a high HSPA2 expression was assigned to all cases stained strongly and moderately. In the univariate model, only clinical stage significantly influenced patients' survival (p=0.030). A high HSPA2 expression did not affect OS (HR 1.43, 95% CI 0.84-2.44, p=0.185) in the whole group. When the patients were divided according to clinical stage, we observed an association between high HSPA2 expression and worse survival in the stage I-II subgroup (n=49, median OS, 16.10 *versus* 42.60 months, p=0.015; HR 2.73, 95% CI 1.26-5.94, p=0.011). A small group size (n=32) and limited number of cases with high HSPA2 expression (n=7) did not allow for the analysis in stage III subgroup. No statistically significant association was found when patients were stratified depending on histology, sex and age. In the multivariate model, however, a high HSPA2 expression (HR 2.12, 95% CI 1.17-3.83, p=0.013), together with IIIA stage (p=0.004) and male sex (p=0.021), were associated with an increased risk of death in the whole group (Table IV). In addition, after stratification of the patients according to stage, histology, sex or age, multivariate analysis revealed that high HSPA2 expression was a negative prognostic factor in stage I-II, SCC, males and older age (>58 years) patient subgroups (data not shown).

Table II. *Clinicopathological characteristics of lung cancer patients (n=85).*

	Total
Diagnosis	
Squamous cell carcinoma	56
Adenocarcinoma	29
Stage	
IB	27
IIB	24
IIIA	34
Gender	
Men	73
Women	12
Age: mean±SD (range)	58±7.30 (43-76)
Smoking status	
Smokers	71
Non-smokers	14
Pack-years[a]: mean±SD (range)	36.83±22.42 (2-120)

[a]For smokers only. SD: Standard deviation.

Discussion

Expression of HSPA2 in human primary tumors. The main finding of our TMA survey is the disclosure that HSPA2 can be expressed in various histological types of primary human tumors. Although very small groups of cases were analyzed, our results noticeably expand current knowledge on HSPA2 in tumors. So far HSPA2 was detected only in primary NSCLC (16), bladder TCC (17) and breast cancer (14). Published data also show that the HSPA2 protein is not present in normal tissues but is highly and specifically overexpressed in cancer cells. Such a tumor-specific activation of HSPA2 expression was demonstrated experimentally in bladder TCC and breast tumors (17, 14). However comparison of the published data on the HSPA2 expression in normal tissues (11, 14, 17) with data on its expression in tumors presented in this work suggests that cancer-specific activation of the HSPA2 expression could pertain, beside breast (non-specific IDC and ILC) and bladder TCC also to many other tumor types. Notably, HSPA2 was not found in normal pancreas, liver and prostate tissues (11) but it can be detected in pancreatic AC, liver AC, prostate AC.

Importantly, our results also suggest that a cancer-specific activation of the HSPA2 expression is not a universal phenomenon. It was shown that HSPA2 is expressed in epidermis specifically in basal cells (11). Herein we found that HSPA2 is highly and frequently expressed in skin SCC. The generally accepted view is that skin SCC originates from progenitor cells located in the basal layer of the epithelium (20, 21). Therefore, it can be presumed that in skin SCC (and

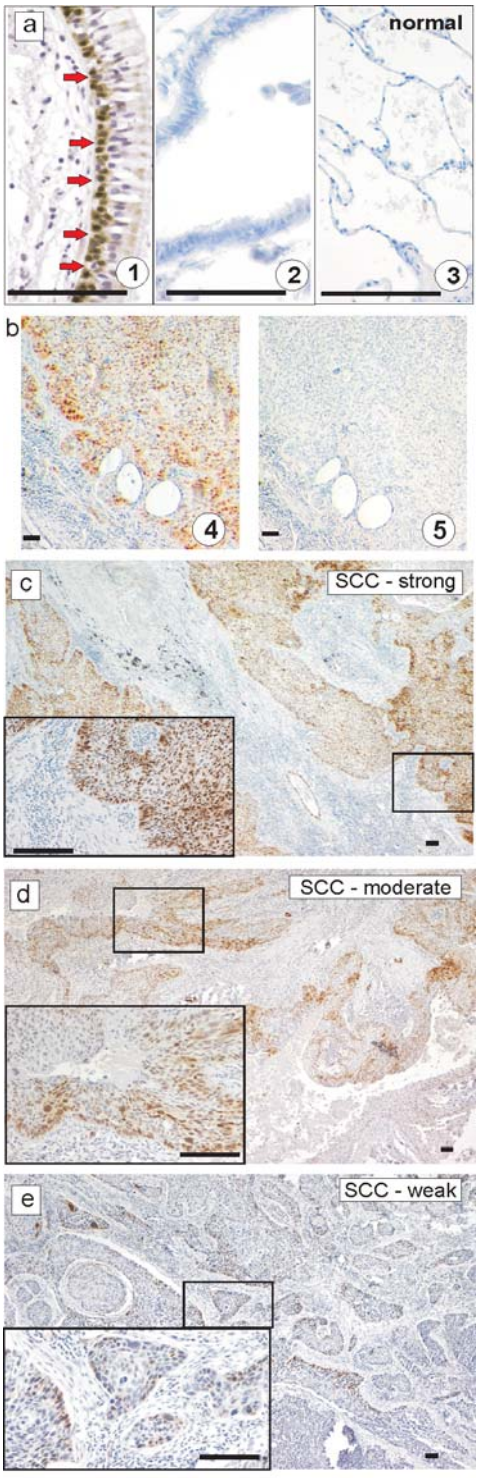

Figure 2. *Detection of HSPA2 in normal lung and in lung SCC. a: Sections of normal bronchial (1), bronchiolar (2) and lung alveolar (3) tissues stained with anti-HSPA2 antibody. Red arrows point at basal cells from ciliated pseudostratified bronchial epithelium. b: Specificity of HSPA2 detection – example of strong HSPA2 staining (4) and results of peptide competition reaction (5). c-d: Examples of strong (c), moderate (d) and weak (e) HSPA2 staining in lung SCC specimens. Bar: 100 μm.*

Table III. *Status of HSPA2 immunoreaction in NSCLC (n=85).*

	HSPA2[a]
Intracellular localization	
Nucleus	35 (41.18)
Nucleus/cytoplasm	18 (21.18)
Cytoplasm	1 (1.18)
IHC scores in NSCLC (n=85)	
Nuclear staining	53 (62.35)
Strong (+3)	15 (17.65)
Moderate (+2)	20 (23.53)
Weak (+1)	18 (21.18)
IHC scores in SCC (n=56)	
Nuclear staining	41 (73)[b]
Strong (+3)	14 (25)
Moderate (+2)	14 (25)
Weak (+1)	13 (13)
IHC scores in AC (n=29)	
Nuclear staining	12 (41)[b]
Strong (+3)	1 (3)
Moderate (+2)	6 (21)
Weak (+1)	5 (17)

NSCLC: Non-small cell lung carcinoma; SCC: squamous cell carcinoma; AC: adenocarcinoma. [a]Data are given as percentages. [b]Statistically significant, SCC *vs.* AC ($p<0.01$).

Table IV. *Multivariate Cox regression analysis of OS in 81 NSCLC patients.*

	HR (95%CI)	*p*-Value
HSPA2 expression		
High	2.12 (1.17-3.83)	0.013
Gender		
Male	2.98 (1.18-7.51)	0.021
Age[a]	1.00 (0.97-1.04)	0.861
Clinical stage		
IIIA	2.16 (1.29-3.64)	0.004
Histology		
SCC	0.59 (0.31-1.11)	0.101
Smoking (pack-years)[a]	0.99 (0.98-1.01)	0.296

[a]Continuous variable.

possibly also in other cancers) HSPA2 expression is not affected by oncogenic transformation and can be retained in cancer cells.

Prognostic significance of HSPA2 protein. Herein, for the first time it has been shown that HSPA2 may have certain prognostic significance in resected NSCLC. To the best of our knowledge, an association between NSCLC patients survival and HSPA2 expression has not been presented before. We found that a high HSPA2 expression is associated with shorter survival of patients in early stage (IB and IIB) NSCLC. Unfortunately, due to a small group size and limited number of cases with high HSPA2 expression we were unable to perform survival analysis in the stage III subgroup. In multivariate analysis high HSPA2 expression together with IIIA stage and male sex were associated with shorter OS in the whole group. At present our results cannot be compared to others for NSCLC patients. However recently it has been shown that elevated HSPA2 expression is associated with shorter OS in a group of 120 esophageal SCC patients. Overexpression of HSPA2 was found to be an independent prognostic factor for esophageal SCC patients (22). We are aware that a serious limitation of our study is the small group of NSCLC patients available for analysis. The small population size raises concerns over how reliable the findings are. Therefore, an extended IHC survey performed on a larger group of NSCLC patients should be performed to confirm the prognostic significance of HSPA2 in NSCLC.

While the HSPA2 expression was associated with poor prognosis in the present study, earlier studies on the same NSCLC group revealed that expression of HSPA1 protein, another member of the HSPA family, was associated with increased patients' survival (23). Hence, it would be interesting to determine whether opposite prognostic effects found for HSPA1 and HSPA2 reflect the functional dissimilarity of each of these proteins in NSCLC. Although the functional diversity among HSPA2 and HSPA1 was suggested by *in vitro* experiments, this issue is still highly debatable (14, 24, 25).

A correlation between overexpression of HSPA2 and worst survival of HSP2-positive NSCLC patients can be explained, at least partially, by an important cancer-related function of HSPA2. The published evidence strongly suggests that HSPA2 is crucial for growth and proliferation of breast and bladder cancer cell lines (14, 17). Also, HSPA2 depletion reduced motility and invasiveness of urothelial cancer cell lines (17). However, at present the biological function of HSPA2 in lung cancer cells has not been evaluated.

We found that HSPA2 is strongly and frequently expressed in SCC compared to AC. For SCC patients high HSPA2 expression was more frequent in the early stage (IB + IIB) than in advanced stage (IIIA) subgroup. Interestingly, for AC cases no association with TNM staging was detected. The lung AC and SCC are two types of NSCLC characterized by different etiology (SCC are more frequent in smokers), histology (SCC are found mostly in the central airways, while AC in the peripheral ones) and putative cellular origin (26). At present, it is considered (26, 27) that lung SCC arises from progenitor cells present in the basal layer of central airway epithelium, whereas AC originates from stem cells, which reside in distal airway-alveolar epithelium (*e.g.* bronchioalveolar stem cells or alveolar epithelial type-2 cells). Therefore, in NSCLC, two scenarios

for changes of the HSPA2 expression can be proposed. In SCC, tumor cells presumably retain the HSPA2 expression already observed in basal cells of normal bronchial epithelium, however this activity can be repressed during SCC progression. Oppositely, in the lung AC, the *HSPA2* gene seems to be activated during carcinogenesis as no HSPA2-positive cells can be detected in normal bronchioles and alveoli (Figure 2). Thus, referring to these two circumstances, the problem which needs to be addressed in the future is whether the functions of HSPA2 are equivalent in lung SCC and AC. Also the mechanisms behind the *HSPA2* gene repression in tumors are elusive at present. However, it can be speculated that during tumor progression, the *HSPA2* gene becomes silenced *e.g.*, by promoter methylation. Such a supposition is justified by findings showing that the *HSPA2* gene is methylated in cervical, endometrial and bladder cancers (28-30).

Acknowledgements

We thank Ms. K. Klyszcz and Ms. U. Boyko for skillful technical assistance. This work was supported by a grant (NN401 683740) from the National Science Centre, Poland.

References

1 Ciocca DR, Arrigo AP and Calderwood SK: Heat shock proteins and heat shock factor 1 in carcinogenesis ad tumor development: an update. Arch Toxicol *87*: 19-48, 2013.

2 Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B and Hightower LE: Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones *14*: 105-111, 2009.

3 Kalmar B and Greensmith L: Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev *61*: 310-318, 2009.

4 Krawczyk Z, Wisniewski J and Biesiada E: A rat testis-specific hsp70 gene-related transcript is coded by a novel gene from the hsp70 multigene family. Acta Biochim Pol *35*: 377-385, 1988.

5 Zakeri ZF, Wolgemuth DJ and Hunt CR: Identification and sequence analysis of a new member of the mouse HSP70 gene family and characterization of its unique cellular and developmental pattern of expression in the male germ line. Mol Cell Biol *8*: 2925-2932, 1988.

6 Dix DJ, Allen JW, Collins BW, Mori C, Nakamura N, Poorman-Allen P, Goulding EH and Eddy EM: Targeted gene disruption of Hsp70-2 results in failed meiosis, germ cell apoptosis, and male infertility. Proc Natl Acad Sci USA *93*: 3264-3268, 1996.

7 Dix DJ, Allen JW, Collins BW, Poorman-Allen P, Mori C, Blizard DR, Brown PR, Goulding EH, Strong BD and Eddy EM: HSP70-2 is required for desynapsis of synaptonemal complexes during meiotic prophase in juvenile and adult mouse spermatocytes. Development *124*: 4595-4603, 1997.

8 Zhu D, Dix DJ and Eddy EM: HSP70-2 is required for CDC2 kinase activity in meiosis I of mouse spermatocytes. Development *124*: 3007-3014, 1997.

9 Bonnycastle LL, Yu CE, Hunt CR, Trask BJ, Clancy KP, Weber JL, Patterson D and Schellenberg GD: Cloning, sequencing, and mapping of the human chromosome 14 heat shock protein gene (HSPA2). Genomics *23*: 85-93, 1994.

10 Scieglińska D, Widłak W, Rusin M, Markkula M and Krawczyk Z: Expression of the testis-specific HSP70-related gene (hst70 gene) in somatic non-testicular rat tissues revealed by RT-PCR and transgenic mice analysis. Cell Biol Int *21*: 813-21, 1997.

11 Scieglinska D, Piglowski W, Chekan M, Mazurek A and Krawczyk Z: Differential expression of HSPA1 and HSPA2 proteins in human tissues; tissue microarray-based immunohistochemical study. Histochem Cell Biol *135*: 337-350, 2011.

12 Hageman J, van Waarde MA, Zylicz A, Walerych D and Kampinga HH: The diverse members of the mammalian HSP70 machine show distinct chaperone-like activities. Biochem J *435*: 127-142, 2011.

13 Filipczak PT, Piglowski W, Glowala-Kosinska M, Krawczyk Z and Scieglinska D: HSPA2 overexpression protects V79 fibroblasts against bortezomib-induced apoptosis. Biochem Cell Biol *90*: 224-31, 2012.

14 Rohde M, Daugaard M, Jensen MH, Helin K, Nylandsted J and Jäättelä M: Members of the heat-shock protein 70 family promote cancer cell growth by distinct mechanisms. Genes Dev *19*: 570-582, 2005.

15 Piglowski W, Nowak R, Krawczyk Z and Scieglinska D: The structural and functional analysis of the human HSPA2 gene promoter region. Acta Biochim Pol *54*: 99-106, 2007.

16 Scieglinska D, Pigłowski W, Mazurek A, Małusecka E, Zebracka J, Filipczak P and Krawczyk Z: The HspA2 protein localizes in nucleoli and centrosomes of heat shocked cancer cells. J Cell Biochem *104*: 2193-2206, 2008.

17 Garg M, Kanojia D, Seth A, Kumar,R, Gupta A, Surolia A and Suri A: Heat-shock protein 70-2 (HSP70-2) expression in bladder urothelial carcinoma is associated with tumour progression and promotes migration and invasion. Eur J Cancer *46*: 207-215, 2010.

18 Garg M, Kanojia D, Saini S, Suri S, Gupta A, Surolia A and Suri A: Germ cell-specific heat shock protein 70-2 is expressed in cervical carcinoma and is involved in the growth, migration, and invasion of cervical cells. Cancer *116*: 3785-3796, 2010.

19 Malusecka E, Zborek A, Krzyzowska-Gruca S and Krawczyk Z: Immunohistochemical detection of the inducible heat shock protein hsp70: a methodological study. J Histochem Cytochem *54*: 183-190, 2006.

20 Lapouge G, Youssef KK, Vokaer B, Achouri Y, Michaux C, Sotiropoulou PA and Blanpain C: Identifying the cellular origin of squamous skin tumors. Proc Natl Acad Sci USA *108*: 7431-7436, 2011.

21 White AC, Tran K, Khuu J, Dang C, Cui Y, Binder SW and Lowry WE: Defining the origins of Ras/p53-mediated squamous cell carcinoma. Proc Natl Acad Sci USA *108*: 7425-7430, 2011.

22 Zhang H, Chen W, Duan CJ, Zhang CF: Overexpression of HSPA2 is correlated with poor prognosis in esophageal squamous cell carcinoma. World J Surg Oncol *11*: 141, 2013.

23 Malusecka E, Krzyzowska-Gruca S, Gawrychowski J, Fiszer-Kierzkowska A, Kolosza Z and Krawczyk Z: Stress proteins HSP27 and HSP70i predict survival in non-small cell lung carcinoma. Anticancer Res *28*: 501-506; 2008.

24 Kirkegaard T, Roth AG, Petersen NH, Mahalka AK, Olsen OD, Moilanen I, Zylicz A, Knudsen J, Sandhoff K, Arenz C, Kinnunen PK, Nylandsted J and Jäättelä M: Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology. Nature *463*: 549-553, 2010.

25 Daugaard M, Rohde M and Jäättelä M: The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. FEBS Lett *581*: 3702-3710, 2007.

26 Gazdar AF and Brambilla E: Preneoplasia of lung cancer. Cancer Biomark *9*: 385-396, 2010.

27 Lundin A and Driscoll B: Lung cancer stem cells: Progress and prospects. Cancer Lett *338*: 89-93, 2013.

28 Widschwendter A, Gattringer C, Ivarsson L, Fiegl H, Schneitter A, Ramoni A, Müller HM, Wiedemair A, Jerabek S, Müller-Holzner E, Goebel G, Marth C and Widschwendter M: Analysis of aberrant DNA methylation and human papillomavirus DNA in cervicovaginal specimens to detect invasive cervical cancer and its precursors. Clin Cancer Res *10*: 3396-3400, 2004.

29 Fiegl H, Gattringer C, Widschwendter A, Schneitter A, Ramoni A, Sarlay D, Gaugg I, Goebel G, Müller HM, Mueller-Holzner E, Marth C and Widschwendter M: Methylated DNA collected by tampons – a new tool to detect endometrial cancer. Cancer Epidemiol Biomarkers Prev *13*: 882-888, 2004.

30 Costa VL, Henrique R, Danielsen SA, Duarte-Pereira S, Eknaes M, Skotheim RI, Rodrigues A, Magalhães JS, Oliveira J, Lothe RA, Teixeira MR, Jerónimo C and Lind GE: Three epigenetic biomarkers, GDF15, TMEFF2, and VIM, accurately predict bladder cancer from DNA-based analyses of urine samples. Clin Cancer Res *16*: 5842-5851, 2010.

Received February 25, 2014
Revised April 23, 2014
Accepted April 24, 2014