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Expression of heat shock proteins *HSPA1A, HSPA1B* and *TP53* in vulval lichen planus and vulval lichen sclerosus

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ABSTRACT

Objectives: Heat shock proteins (HSPs) are proteins involved in protein folding and maturation. HSP expression is induced by heat shock or other stressors including cellular damage and hypoxia. The major groups, which are classified based on their molecular weight, include HSP27, HSP40, HSP60, HSP70, HSP90, and large HSP (HSP110 and glucose-regulated protein 170). The comparison of heat shock proteins and *TP53* expression is yet not well studied in both vulval lichen sclerosus and lichen planus. Our aim was to assess the HSP and *TP53* gene expression in women suffering from LS or LP and compare it within these groups and also healthy controls.

Material and methods: The inclusion criteria were willingness to donate vulval biopsies, not currently or in the prior two weeks received any local nor systemic treatment for vulval disorder, age > 18 years old. The exclusion criteria were lack of consent, current vaginal infection confirmed with microbiological studies, current local or systemic treatment for

vulval disease. 45 consecutive women were recruited into the study. All appropriate vulval samples were process by genetic analysis.

Results: The mean expression (± SD) of *HPSA1A* for controls was 5.52 ± 3.18, for LS was 7.44 ± 2.16 and for LP was 7.89 ± 2.48. The mean expression (± SD) of *HPSA1B* for controls was 6.54 ± 3.41, for LS was 9.94 ± 6.88 and for LP was 9.43 ± 2.31. The mean expression (± SD) of *TP53* for controls was 9.11 ± 1.14, for LS was 9.94 ± 1.27 and for LP was 10.41 ± 2.00. *HSPA1A* expression was 3,8 higher in women with lichen sclerosus than in control group.

Conclusions: Heat shock protein-70 is more often expressed in LS than in healthy controls. HSP-70 not only supports tumor growth and metastasis, but on the other hand mat help to develop immune-driven treatment strategies.

Key words: heat shock proteins; TP53; lichen sclerosus; lichen planus; vulva

INTRODUCTION

Heat shock proteins (HSPs) are proteins involved in protein folding and maturation. HSP expression is induced by heat shock or other stressors including cellular damage and hypoxia. The major groups, which are classified based on their molecular weight, include HSP27, HSP40, HSP60, HSP70, HSP90, and large HSP (HSP110 and glucose-regulated protein 170) [1]. Heat shock protein-70 is a stress-inducible 70-kDa molecular chaperone of the highly conserved HSP70 family [2]. The main physiologic functions of HSP70 include: folding native and denaturated proteins to native state, refolding of intracellular aggregated proteins, degradation of aggregated proteins by ubiquitin proteosomes system or chaperones-mediated autophagy [3]. The family of heat shock proteins 70-kDa is constituted by member 1A and 1B (HSPA1A and 1B) [4, 5]. In normal unstressed cells, HSP70 is expressed at low or undetectable levels [3]. HSP70 has cytoprotective role for different types of cell death like apoptosis, necrosis and autophagic cell-programmed death [6, 7]. The expression of inducible HSP70 in the membrane of various tumor cells, but not normal cells, was reported by several studies in response to some stressful conditions [8]. This direct involvement of HSP70 in most of the cancer hallmarks explains the phenomenon of cancer “addiction” to HSP70, tightly linking tumor survival and growth to the HSP70 expression [9]. HSP70 plays a critical role in occurrence, progression and cancer metastasis [10–12]. Our group previously explored the role of heat shock proteins in ovarian cancer [13]. We compared women with ovarian cancer

and healthy controls finding significantly elevated levels of heat shock protein (HSP 27) in women with ovarian cancer [13].

The most important part in cancer prevention lies in defining the precancerous state linked to cell changes favoring progression into cancer cells. Both lichen sclerosus (LS) and lichen planus (LP) are little studied, however its transformation to vulvar cancer was previously described [14, 15]. These are two debilitating skin disorders that often affect women's genital area. Both are chronic and inflammatory diseases, often difficult to treat with high recurrence [16, 17]. The prevalence of vulval lichen sclerosus is unknown due to underdiagnosis and referral bias, but it has been reported to constitute about 10% of new cases in vulval practice [18]. When assessing the severity of the disease the following elements should be considered per Australasian management consensus: fusion, lichenification, loss of vulval architecture, extent of disease, ulceration and purpura [18]. Vulval lichen planus has a broad clinical presentation, ranging from diffuse erythema to severe erosions or hyperkeratotic plaques, with possible scarring and loss of vulval architecture [14]. Boch et al. [14] reported recently that the incidence rate of vulval squamous cell carcinoma in women with vulval lichen planus is 25.9 per 1000 person-years. It has been shown that epithelial HSP 70 staining intensity is greater in oral and cutaneous lichen planus lesions than in normal oral mucosa and skin [19, 20]. p53 is a 53 kDa protein that responds to DNA damage by stalling the cell cycle at G1 to facilitate DNA repair [20]. Inactivation of p53 is frequently in carcinogenesis and *TP53* gene mutation is implicated in this mechanism [21]. Previously, *TP53* overexpression in LS was found to be related with squamous vulvar carcinoma and in patients with long- standing LS, the TP53 expression is higher than in short-term LS [21]. The data on TP53 expression in vulval lichen planus is limited. One study described p53 staining in vulval tissue in three patients. One showed strong staining of 80% of basal cells extending into two-third of the epithelial thickness. The other patients had only 10% of basal cells stained, extending into one-third of epithelial thickness. Thus, this report does not show a consistent trend within lichen planus samples [22].

Objectives

The comparison of heat shock proteins and *TP53* expression is yet not well studied in both vulval lichen sclerosus and lichen planus. Our aim was to assess the HSP and *TP53* gene expression in women suffering from LS or LP and compare it with each other and healthy controls.

MATERIAL AND METHODS

The study was approved by Ethical Committee (Medical University of Silesia, Poland). Women with suspected vulval lichen planus or lichen sclerosus who were admitted to the Department of Gynecology, Obstetrics and Oncological Gynecology for vulval biopsy or women who undergone surgery for uterus disease (as control group) were invited to participate in the study. The inclusion criteria were: willingness to donate vulval biopsies, not currently or in the prior two weeks received any local nor systemic treatment for vulval disorder, age > 18 years old. The exclusion criteria were lack of consent, current vaginal infection confirmed with microbiological studies, current local or systemic treatment for vulval disease. 45 consecutive women were recruited into the study.

The vulval biopsies were taken from areas suspected of disease or in case of controls from macroscopically healthy vulva at 5 and 8 o'clock by an experienced gynecologist in vulval disease (IG) under local anesthesia with 1% lidocaine. Vulval biopsies were immediately divided into two probes for standard pathological analysis to confirm the diagnosis and for genetical analysis.

Genetic analysis

Biopsied vulval tissue approximately 3-5 mm in length were collected from patients and immediately immersed in RNAlater RNA stabilizing reagent (Qiagen, Germany).

Total RNA was isolated using RNeasyMini Kit (Qiagen, Germany) following sample homogenization in TissueLyser II (Qiagen, Venlo, The Netherlands). During extraction, RNA was treated with deoxyribonuclease I (Qiagen) to avoid DNA contamination. Quality and quantity evaluation were performed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). Total RNA (0.5 to 2 µg) was transcribed using a cDNA Transcriptor First Strand cDNA Synthesis Kit (Roche, Penzberg, Upper Bavaria, Germany) using Random Hexamers. Expression analyses with Real Time Custom Panel 96-16 (config. no 100118810; Roche, Penzberg, Upper Bavaria, Germany) and LightCycler 480 Probe Master (Roche, Germany) were performed in duplicates using a LightCycler 480 II (Roche). The genes analyzed in this report are listed in Table 1.

Table 1. List of analyzed genes in alphabetical order, two reference genes at the beginning of the table

	Assay ID	Gene Symbol	Alias	Description
	141139	*GAPDH* H. sapiens	G3PD, GAPD, MGC88685	Glyceraldehyde-3-phosphate dehydrogenase [Source:HGNC Symbol;Acc:4141]
	104092	*RN18S1* H. sapiens		RNA, 18S ribosomal 1
10	145875	*HSPA1A* H. sapiens	FLJ54303, FLJ54370, FLJ54392, FLJ54408, FLJ75127, HSP70-1, HSP70-1A, HSP70I, HSP72, HSPA1, HSPA1B	Heat shock 70kDa protein 1A [Source:HGNC Symbol;Acc:5232]
11	100490	*HSPA1B* H. sapiens	FLJ54328, HSP70-1B, HSP70-2, HSPA1A	Heat shock 70 kDa protein 1A/1B (Heat shock 70 kDa protein 1/2)(HSP70.1/HSP70.2) (HSP70-1/HSP70-2) [Source:UniProtKB/Swiss-Prot;Acc:P08107]
14	101277	*TP53* H. sapiens	FLJ92943, LFS1, p53, TRP53	tumor protein p53 [Source:HGNC Symbol;Acc:11998]

Gene expression profiling

Gene expression was analyzed using GenEx ver6 software (MultiD analyses AB; Göteborg, Sweden). Data were analyzed using relative expression method - ΔCt. Raw data were subjected to normalization to average technical repeats followed by normalization to sample amount and the reference genes *GAPDH*, *RN18S1* (Tab. 1). The last preprocessing step was filling the missing data with 0.

Statistical analysis

Kolmogorov-Smirnov test was employed to determine if the data on the population show normal distribution. As the data were not normally distributed the non-parametric test:

Mann-Whitney 1-tail test was engaged. The threshold for p-value was set less than 0.05. Spearman correlation coefficient (R_S) was calculated to determine the correlation between genes.

RESULTS

Thirty-nine patients were included in the final analysis with the remaining 6 excluded due to small tissue portion not enabling further genetic assessment (n = 4) or pathologic diagnosis of vulvar hyperkeratosis in control patients (n = 2). Ten patients had lichen planus, 15 had lichen sclerosus and 14 were in control group. The age of patients ranged from 24 years old to 75 years old, with 58% of them being menopausal. The mean expression (± SD) of *HPSA1A* for controls was 5.52 ± 3.18, for LS was 7.44 ± 2.16 and for LP was 7.89 ± 2.48. The mean expression (± SD) of *HPSA1B* for controls was 6.54 ± 3.41, for LS was 9.94 ± 6.88 and for LP was 9.43 ± 2.31. The mean expression (± SD) of *TP53* for controls was 9.11 ± 1.14, for LS was 9.94 ± 1.27 and for LP was 10.41 ± 2.00.

In Table 2, we compared heat shock proteins and *TP53* gene expression between LP vs controls, LS vs controls and LP vs LS. We found significant difference in *HSPA1A* expression between patients in control group and those with lichen sclerosus. *HSPA1A* expression was 3.8 higher in women with lichen sclerosus than in control group. Similar pattern of expression was observed for *HSP1B* but there was no statistical significance between groups. Expression of the *TP53* gene in patients with LS and LP did not differ statistically in comparison with the control group.

Table 2. Fold change of gene expression between lichen sclerosus (LS), lichen planus (LP) and controls normalized with two reference genes

	Control vs LP	Controls vs LS	LP vs LS
HSPA1A	–5.17812	-3.80267*	1.36171
HSPA1B	-7.38486	-10.52918	-1.42578
TP53	-2.4701	-1.789	1.38072

*Statistical differences for p > 0.05. Genes (*HSPA1A*, *HSPA1B*, *TP53*), when correlated between each other showed significant strong correlations: *HSPA1A* and *HSPA1B* (Rs = 0.74; p = 0.00), *HSPA1A* and *TP53* (Rs = 0.71; p = 0.00) and HSPA1B and *TP53* (Rs = 0.69; p = 0.03)

DISCUSSION

Our study reports novel findings on the genetics related to lichen sclerosus and lichen planus. The expression of heat shock proteins (*HSPA1A*, *HSPA1B*) known as HSP70 is higher in patients with LS and LP than in healthy controls. However, the statistical difference was only observed for *HSPA1A* between LS patients and healthy women. This is probably due to a small sample size, thus we encourage further studies in this important topic. *TP53* expression was non-different between lichen planus, lichen sclerosus and healthy controls or between each other.

It is the first study to analyze HSP70 expression in vulval lichen planus, as the previous studies either investigated the intensity of immunostaining in cutaneous LP biopsies (biopsies taken from other body areas) [22] or were limited to oral biopsies [23]. Previously, the expression of HSP70 was found to be non-different between different types of lichen planus (classical, eruptive, hypertrophic, planopilaris) [23]. Bayramgurler et al. [23] observed based on immunohistochemical evaluation with IRIDI (immunoreactivity intensity distribution index) score, a significantly lower HSP70 expression in basal layer of LP skin than normal skin, with non-different expression in suprabasal and superficial epidermal layers. In our study, which was based on genetic analysis of vulval tissue, we found no difference in expression of HSP70 between LP and healthy controls samples. Similarly, we found no difference between LP and LS samples. There is no previous literature about heat shock protein in lichen sclerosus, so we are unable to compare our findings with any former data. *HSPA1A* expression was higher in LS specimen than in controls, which may partially explain that a small percentage of patients with LS who develop squamous vulvar cancer [16]. Cancer cells are dependent on chaperones (among them HSP70) and constitutively upregulate their expression to inhibit cell apoptosis and promote abnormal cell growth [25]. HSP 70 was shown to be crucial for the survival of tumor cells [3] and heat shock proteins may contribute in different ways to transformation and tumor progression, depending on the driver oncogene that mediates tumorigenesis [25]. The role of heat shock proteins was previously shown in cervical cancer [26]. Both RT-PCR and immunohistochemical staining for cervical cancer cells presented an increasing presence of HSP70-2 which was associated with stage of cervical cancer (60% of IA specimens, 77% of IB specimens, 90% of IIA specimens and 100% of IIB specimens). Another heat shock protein (*HSP90*) and *TP53* were evaluated in oral lichen planus (OLP) and oral squamous cell cancer (OSCC). In over 100 patients, the increasing expression was shown in OLP and OSCC, 50% and 72% respectively [27]. T*P53* was expressed in 32% of LP and 45% of OSCC [27]. In vulvar tissue, moderate to strong *p53*

staining was shown in lichen sclerosus (7 cases) and in lichen planus (1 case) [22]. As mentioned previously, only a minority of LS cases is associated with squamous vulvar carcinoma [21], however a previous study presented an increasing *TP53* expression from control to LS and squamous vulvar cancer which correlated with tumor progression [28]. In our study, *TP53* expression was non- different between the groups. Thus, we suggested that this topic should be investigated in larger population, as *TP53* over expression was suggested as a feature of vulvar LS that evolved to vulvar cancer. It is noteworthy that all the referenced studies had small group of patients *eg.* the study by Raspollini [29] evaluated only 8 patients.

CONCLUSIONS

In conclusion, our finding that heat shock protein-70 is more often expressed in LS than in healthy controls, should prompt further studies. HSP-70 not only supports tumor growth and metastasis, but on the other hand mat help to develop immune-driven treatment strategies [30, 31].

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Conflict of interest

Authors report no conflict of interest

REFERENCES

1. Aswad A, Liu T. Targeting heat shock protein 90 for anti-cancer drug development. Adv Cancer Res. 2021; 152: 179–204, doi: 10.1016/bs.acr.2021.03.006, indexed in Pubmed: 34353437.
2. Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci. 2005; 62(6): 670–684, doi: 10.1007/s00018-004-4464-6, indexed in Pubmed: 15770419.
3. Ellmalah MIY, Cordonnier M, Vautrot V, et al. Membrane-anchored heat-shock protein 70 (Hsp 70) in cancer. Cancer Lett. 2020; 469: 134–41.
4. HSPA1A heat shock protein family A (Hsp70) member 1A [Homo sapiens (human)]. https://www.ncbi.nlm.nih.gov/gene/3303.

5. HSPA1B heat shock protein family A (Hsp70) member 1B [Homo sapiens (human)]. https://www.ncbi.nlm.nih.gov/gene/3304.

6. Park YH, Seo JiH, Park JH, et al. Hsp70 acetylation prevents caspase-dependent/independent apoptosis and autophagic cell death in cancer cells. Int J Oncol. 2017; 51(2): 573–578, doi: 10.3892/ijo.2017.4039, indexed in Pubmed: 28627586.

7. Srinivasan SR, Cesa LC, Li X, et al. Heat Shock Protein 70 (Hsp70) Suppresses RIP1-Dependent Apoptotic and Necroptotic Cascades. Mol Cancer Res. 2018; 16(1): 58–68, doi: 10.1158/1541-7786.MCR-17-0408, indexed in Pubmed: 28970360.

8. Boudesco C, Cause S, Jego G, et al. Hsp70: A Cancer Target Inside and Outside the Cell. Methods Mol Biol. 2018; 1709: 371–396, doi: 10.1007/978-1-4939-7477-1_27, indexed in Pubmed: 29177673.

9. Albakova Z, Armeev GA, Kanevskiy LM, et al. HSP70 Multi-Functionality in Cancer. Cells. 2020; 9(3), doi: 10.3390/cells9030587, indexed in Pubmed: 32121660.

10. Kabakov AE, Gabai VL. HSP70s in Breast Cancer: Promoters of Tumorigenesis and Potential Targets/Tools for Therapy. Cells. 2021; 10(12), doi: 10.3390/cells10123446, indexed in Pubmed: 34943954.

11. Lei Z, Xia X, He Q, et al. HSP70 promotes tumor progression by stabilizing Skp2 expression in gastric cancer cells. Mol Carcinog. 2021; 60(12): 826–839, doi: 10.1002/mc.23346, indexed in Pubmed: 34499769.

12. Kasioumi P, Vrazeli P, Vezyraki P, et al. Hsp70 (HSP70A1A) downregulation enhances the metastatic ability of cancer cells. Int J Oncol. 2019; 54(3): 821–832, doi: 10.3892/ijo.2018.4666, indexed in Pubmed: 30569142.

13. Bodzek P, Damasiewicz-Bodzek A, Janosz I, et al. Heat shock protein 27 (HSP27) in patients with ovarian cancer. Ginekol Pol. 2021; 92(12): 837–843, doi: 10.5603/GP.a2021.0061, indexed in Pubmed: 34105741.

14. Boch K, Langan EA, Zillikens D, et al. Retrospective analysis of the clinical characteristics and patient-reported outcomes in vulval lichen planus: Results from a single-center study. J Dermatol. 2021; 48(12): 1913–1917, doi: 10.1111/1346-8138.16191, indexed in Pubmed: 34668211.

15. Pérez-López FR, Vieira-Baptista P. Lichen sclerosus in women: a review. Climacteric. 2017; 20(4): 339–347, doi: 10.1080/13697137.2017.1343295, indexed in Pubmed: 28661705.

16. Fistarol SK, Itin PH. Diagnosis and treatment of lichen sclerosus: an update. Am J Clin Dermatol. 2013; 14(1): 27–47, doi: 10.1007/s40257-012-0006-4, indexed in Pubmed: 23329078.

17. Simpson RC, Thomas KS, Leighton P, et al. Diagnostic criteria for erosive lichen planus affecting the vulva: an international electronic-Delphi consensus exercise. Br J Dermatol. 2013; 169(2): 337–343, doi: 10.1111/bjd.12334, indexed in Pubmed: 23521206.

18. Yeon J, Oakley A, Olsson A, et al. Vulval lichen sclerosus: An Australasian management consensus. Australas J Dermatol. 2021; 62(3): 292–299, doi: 10.1111/ajd.13594, indexed in Pubmed: 34117779.

19. Tavassol F, Starke OF, Völker B, et al. Heat-shock protein expression and topical treatment with tacrolimus in oral lichen planus: an immunohistochemical study. Int J Oral Maxillofac Surg. 2008; 37(1): 66–69, doi: 10.1016/j.ijom.2007.06.016, indexed in Pubmed: 17822876.

20. Bayramgürler D, Ozkara SK, Apaydin R, et al. Heat shock proteins 60 and 70 expression of cutaneous lichen planus: comparison with normal skin and psoriasis vulgaris. J Cutan Pathol. 2004; 31(9): 586–594, doi: 10.1111/j.0303-6987.2004.00234.x, indexed in Pubmed: 15330989.

21. Carlson BC, Hofer MD, Ballek N, et al. Protein markers of malignant potential in penile and vulvar lichen sclerosus. J Urol. 2013; 190(2): 399–406, doi: 10.1016/j.juro.2013.01.102, indexed in Pubmed: 23399649.

22. Liu YA, Ji JX, Almadani N, et al. Comparison of p53 immunohistochemical staining in differentiated vulvar intraepithelial neoplasia (dVIN) with that in inflammatory dermatoses and benign squamous lesions in the vulva. Histopathology. 2021; 78(3): 424–433, doi: 10.1111/his.14238, indexed in Pubmed: 32799363.

23. Bayramgürler D, Ozkara SK, Apaydin R, et al. Heat shock proteins 60 and 70 expression of cutaneous lichen planus: comparison with normal skin and psoriasis vulgaris. J Cutan Pathol. 2004; 31(9): 586–594, doi: 10.1111/j.0303-6987.2004.00234.x, indexed in Pubmed: 15330989.

24. Sugerman PB, Savage NW, Xu LJ, et al. Heat shock protein expression in oral lichen planus. J Oral Pathol Med. 1995; 24(1): 1–8, doi: 10.1111/j.1600-0714.1995.tb01121.x, indexed in Pubmed: 7722915.

25. Calderwood SK, Gong J. Heat Shock Proteins Promote Cancer: It's a Protection Racket. Trends Biochem Sci. 2016; 41(4): 311–323, doi: 10.1016/j.tibs.2016.01.003, indexed in Pubmed: 26874923.

26. Garg M, Kanojia D, Saini S, et al. 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. 2010; 116(16): 3785–3796, doi: 10.1002/cncr.25218, indexed in Pubmed: 20564126.

27. Bar JK, Cierpikowski P, Lis-Nawara A, et al. Comparison of p53, HSP90, E-cadherin and HPV in oral lichen planus and oral squamous cell carcinoma. Acta Otorhinolaryngol Ital. 2021; 41(6): 514–522, doi: 10.14639/0392-100X-N1450, indexed in Pubmed: 34928263.

28. Sadalla JC, Lourenço SV, Sotto MN, et al. Claudin and p53 expression in vulvar lichen sclerosus and squamous-cell carcinoma. J Clin Pathol. 2011; 64(10): 853–857, doi: 10.1136/jclinpath-2011-200103, indexed in Pubmed: 21642657.

29. Raspollini MR, Asirelli G, Moncini D, et al. A comparative analysis of lichen sclerosus of the vulva and lichen sclerosus that evolves to vulvar squamous cell carcinoma. Am J Obstet Gynecol. 2007; 197(6): 592.e1–592.e5, doi: 10.1016/j.ajog.2007.04.003, indexed in Pubmed: 17714682.

30. Lv LH, Wan YL, Lin Y, et al. Anticancer drugs cause release of exosomes with heat shock proteins from human hepatocellular carcinoma cells that elicit effective natural killer cell antitumor responses in vitro. J Biol Chem. 2012; 287(19): 15874–15885, doi: 10.1074/jbc.M112.340588, indexed in Pubmed: 22396543.

31. Xie Y, Bai Ou, Zhang H, et al. Membrane-bound HSP70-engineered myeloma cell-derived exosomes stimulate more efficient CD8(+) CTL- and NK-mediated antitumour immunity than exosomes released from heat-shocked tumour cells expressing cytoplasmic HSP70. J Cell Mol Med. 2010; 14(11): 2655–2666, doi: 10.1111/j.1582-4934.2009.00851.x, indexed in Pubmed: 19627400.