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Association between p53 protein phosphorylated at serine 20 expression and ovarian carcinoma stem cells phenotype: correlation with clinicopathological parameters of ovarian cancer

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Biological behavior of ovarian carcinomas might be the result of cellular diversity existing in tumor tissue, which consists of differentiated and undifferentiated cells showing stem cells biological properties and function. We examined correlation between p53 protein phosphorylated at serine 20 (p-p53(Ser20)) and CD133, SOX2, Notch1 expression, in order to reveal p-p53(Ser20) stemness function in ovarian cancer. p-p53(Ser20), CD133, Notch1, SOX2 expression was analyzed on 104 ovarian carcinomas using immunohistochemical staining. The positive correlation between p53 and p-p53(Ser20) (p=0.02), p53 and SOX2 (p=0.02), p-p53(Ser20) and Notch1 (p=0.03), p-p53(Ser20) and CD133 (p=0.01) expression was observed in ovarian carcinomas. The parallel expression of p-p53(Ser20)/CD133, p-p53(Ser20)/Notch1 reflecting co-expression of these proteins in single carcinoma cell, and p-p53(Ser20)/SOX2 expression was associated with advanced stage and p-p53(Ser20)/Notch1, p53/SOX2, p-p53(Ser20)/SOX2 parallel expression correlated with high tumor grade. The correlation between p-p53(Ser20) and CD133, Notch1, SOX2 expression and clinical parameters indicate, that malignancy and biological behavior of ovarian carcinomas depend on interaction between p-p53(Ser20) and carcinoma stem cells biomarkers expression.

Key words: ovarian carcinoma, p53 phosphorylation, CSCs biomarkers expression

Growing evidence reveals that the limited effectiveness of therapy in patients with ovarian cancer might be the result of morphological and biological heterogeneity of ovarian cancer [1–4]. It was noticed, that ovarian tumors contain two different fractions of tumor cells; one of them is a differentiated tumor cells, the second one appears in an inappropriately differentiated stage described as a dedifferentiated tumor cells which might cause the chemoresistance of ovarian cancer [1, 3–4]. This subpopulation of ovarian cancer cells often shows properties similar to normal adult stem cells with the innate capacity to self-renew [1, 4]. These cells also possess unique features like the ability to initiate the growth and maintenance of tumor heterogeneity and were described as ovarian carcinoma stem cells (CSCs) [1, 4]. CSCs express specific stem cell markers (e.g. CD133, CD117, CD44, CD24), transcriptional factors (SOX2, OCT4, NANOG), receptors of signaling pathways (Notch, WNT, Hedgehog), which are involved in tumor cell activation and growth [1, 4–7]. Some authors suggested that alterations in oncogenes and suppressor genes, which are observed in ovarian differentiated tumor cells, might occur in CSCs [8–10]. Recently, it was found that p53 has a great impact on processes such as cellular differentiation, self-renewal ensuring a balance between genome stability and plasticity in normal adult stem cells [11–13]. There are data, which show that the activation of p53 inhibits the core transcription factor Nanog, thereby promoting stem cell differentiation in response to DNA and also reduces pluripotent stem cells generation efficiency [13, 14]. Some studies have shown that aberration of p53 expression can promote CSCs initiation and proliferation [5]. There is study, which suggest that p53 functional abnormalities found in mesenchymal stem cells (MSCs) promotes osteosarcoma development [15]. Similarly, p53 abnormalities found in mammary, hematopoietic and neural stem cells could generate aberrant stem cells and promote tumor formation [11, 13]. It was found, that the activation of p53 alone or in association with other factors is capable of inhibiting cancer via inhibition of stem cell-related mechanisms [13–15]. p53

protein plays the suppressive function when it is stabilized through posttranslational modification via phosphorylation, acetylation, methylation and ubiquitination [11–14]. Some authors revealed that active forms of p53 protein are able to inhibit transcription factors Nanog, OCT3/4, Sox2 which are expressed individually or collectively in many cancer cell types [11, 13, 14]. It has also been demonstrated that active p53 protein expressions in stem cells have been associated with Wnt/β catenin signaling pathways, Sonic Hedgehog (Shh), BIM-1, Notch and PTEN pathways that provide balance of self-renewal and differentiation through niche signaling [11, 13]. The function of p53 protein phosphorylation in adult or embryonic stem cells is weakly described [13, 14]. It was found that after DNA damage in embryonic stem cells, p53 binds to the Nanog promoter region and suppresses Nanog expression through serine 213 or serine 315 p53 phosphorylation and promotes stem cells differentiation [11–13]. In mice model has been found, that p53 protein phosphorylated at serine 23 and threonine (Thr) 21 (equivalent to human Thr18, Ser20), shows depletion of multiple stem cell populations, mainly through p53-mediated expression of PUMA [16]. Lately, Kamińska et al. analyzed p53 phosphorylation at serine 15 and 20 in ovarian carcinoma cell lines and cells isolated form ascitic fluid of patients with ovarian cancer and their results revealed that sensitivity to chemotherapy used depend on p53 protein phosphorylation status [17]. Moreover, it was found that p53 protein phosphorylated at Ser15 and Ser20 determined the apoptotic activity of ovarian carcinoma cells after chemotherapy [17]. The mechanisms by which wild-type p53 or mutant p53 influence stemness in non-malignant stem cells and CSCs are poorly understood [13]. Likewise, the role of p53 wild–type protein or mutant protein isoforms phosphorylation in cancer stem cells biology is unclear. Several authors suggest that active mutant p53 protein showed oncogenic properties that are independent of wild type of p53 [11, 13, 14]. There are no data on whether phosphorylation of mutant p53 is necessary to initiate tumor formation by promoting the generation and expansion of pluripotent cells [13, 14]. Up to now, there are no studies on p53 phosphorylation at serine 20 in ovarian carcinoma cells demonstrating a cancer stem–like phenotype. Nothing is known about the interaction between p53 protein phosphorylation at serine 20 (p-p53(Ser20) and stem cells biomarkers expression in relation to biological and clinical features of ovarian cancers.

The aim of this study was to estimate p-p53(Ser20) expression in relation to cancer stem cells biomarkers e.g. CD133, SOX2, Notch1 in order to reveal p-p53(Ser20) protein stemness function in ovarian cancer.

Patients and methods

Patients. One hundred and four patients with primary ovarian carcinoma before therapy were entered in this study between May 2008 and July 2016. Formalin–fixed, paraffin-embedded tumor tissues from patients with ovarian carcinoma were obtained from the First Gynecologic Clinic of Medical University, Wroclaw, Poland. Malignant tumors were staged according to the International Federation of Gynecology and Obstetrics (FIGO) criteria [18]. Nine tumors were in stage I, thirty in II, fifty were in III, fifteen in IV FIGO stage.

Tissue specimens. Ovarian tumor specimens were histologically verified to confirm the histological type and grade of tumor according to earlier established criteria [19]. The study consists of 35 serous, 39 endometrioid, 18 undifferentiated and 12 mucinous ovarian carcinomas. Thirty-two tumors were well (G1), 35 moderately (G2) and 37 poorly (G3) differentiated.

Cell lines. A primary ovarian cell line OvBH-1 with p53 mutation in exon 6 codon 224 change from GA<u>G</u> to GA<u>A</u> (patent PL189880) and cell line SW626 derived from an ovarian metastasis of a primary adenocarcinoma of the colon with p53 mutation in exon 8 codon 262 change from G<u>G</u>T to G<u>T</u>T (ACCC, USA) were used in this study. Cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (Gibco, Karlsruhe, Germany) and supplemented with antibiotics (penicillin/streptomycin; Sigma-Aldrich, Poznan, Poland). The cells were maintained in a humidified atmosphere at 37 °C and 5% CO_2. When cells reached confluence, they were removed by trypsinization (Trypsin 0.25%; Sigma-Aldrich, Poznan, Poland) and used for further experiments. For immunohistochemical staining the cells specimens were fixed 10 min in cold acetone and dry for 20 min at room temperature.

Antibodies. Immunohistochemical staining of the p53 protein was performed with the following antibodies: mouse monoclonal antibody DO-7 (clone 7) reacts with both wild- and mutant forms of unphosphorylated human p53 protein, recognizing an epitope between amino acids 20 and 25 (Novocastra, Newcastle, UK). Rabbit polyclonal antibody phospho-specific p53 (Ser20) detects endogenous levels of the p53 protein phosphorylation at serine 20 (#9287, Cell Signaling Technology, Boston, USA). Rabbit polyclonal anti-CD133 antibody (orb9913, Biorbyt, Cowley, UK), rabbit polyclonal antibody anti-SOX2 transcriptional factor (orb11398, Biorbyt, UK), rabbit monoclonal antibody anti-Notch1 (D1E11) receptor detects intracellular epitopes between 2 400–2 500 amino acids of human Notch1 (#3608, Cell Signaling Technology, Boston, USA).

Immunohistochemistry. Immunohistochemical staining for analyzed proteins was performed on paraffin-embedded ovarian carcinomas tissue and on cytospin preparations of OvBH-1 and SW626 cells using the Universal Dako REAL EnVision Detection System, Peroxidase/DAB+, Rabbit/Mouse (Dako, Copenhagen, Denmark) and primary antibodies: anti-p53 protein, anti-p-p53(Ser20), anti-Notch1, anti-CD133, anti-SOX2. Four-μm sections from one selected block from each ovarian tumor were deparaffinized and boiled 2×5 min in a citrate buffer (pH=6.0) at 800 W

Table 1. The association between expression of total p53 protein, p53, p-p53(Ser20), stem cell markers and clinico-pathological factors of ovarian carcinomas.

Parameters	Immunoreactivity									
	No cases	p53		p-p53(Ser20)		CD133		Notch1		SOX2	
	n	n [%]	p-value	n [%]	p-value	n [%]	p-value	n [%]	p-value	n [%]	p-value
Histological subtype of ovarian cancers											
serous	35	17(48.5)		19(54.2)		12(34.2)		12(34.2)		30(85.7)	
non-serous	69	25(36.2)	NS	27(39.1)	NS	28(40.5)	NS	22(31.8)	NS	53(76.8)	NS
FIGO stages											
I/II	39	15(38.4)		10(25.6)		12(30.7)		9(23.0)		29(74.3)	
III/IV	65	27(41.5)	NS	36(55.3)	0.003	28(43.0)	NS	25(38.4)	NS	54(83.0)	NS
Tumor grade											
G1/G2	67	22(32.8)		22(32.8)		25(37.3)		17(25.3)		50(74.6)	
G3	37	20(54.0)	0.035	24(64.8)	0.016	15(40.5)	NS	17(45.9)	0.032	33(89.1)	0.045

n – number of positive cases. NS – no statically significant.

in a microwave. After the microwave treatment, the tissue sections were slowly cooled for 30 minutes. Nonspecific tissue and endogenous peroxidase reactivity were blocked with Dako REAL Peroxidase Blocking Solution (Dako, Copenhagen, Denmark). Tissue specimens were incubated with primary antibodies overnight at 4 °C. Cells specimens were incubated with primary antibodies 60 min at room temperature. After washing the tissue and cells specimens with 0.1 M Tris-buffer, pH=7.4 (TBS), they were incubated with Dako REAL EnVision/HRP, Rabbit/Mouse (Dako, Copenhagen, Denmark) for 30 min at room temperature. After washing with TBS the antigen-antibody reaction was visualized by DAB (3,3-diaminobenzidine) (Dako, Denmark) as a chromogen. Sections were counterstained with hematoxylin and mounted. The incubation buffer (TBS) without primary antibodies was used as a negative control. Positive controls for each antibody were performed according the manufacturers' recommendation. In selected representative cases of ovarian carcinomas as well as in cell lines double staining for p-p53(Ser20) and CD133, or Notch1 was performed using EnVision DuoFLEX System according to the manufactures' procedure (Dako, Copenhagen, Denmark).

Interpretation of immunohistochemical staining. Assessment of analyzed proteins expression in tumor tissue and cell specimens was scored semiquantitatively, taking into account the intensity of immunostaining and number of tumor cells showing immunoreactivity for analyzed proteins. The number of tumor cells exhibiting staining for p53, p-p53(Ser20), and SOX2 antibodies were assessed by counting 1000 cells in randomly selected 10–15 high power fields. CD133 and Notch1 expression was analyzed by determining cytoplasmic/membrane immunostaining based on the intensity of immunostaining and the percentage of stained tumor tissue area. In cytospin preparation, the percentage of positive cells for p53, p-p53(Ser20), SOX2, CD133, Notch1 proteins expression was determined by counting 1000 cells in randomly selected areas. The cases were scored as negative for p53, p-p53(Ser20) and stem cells markers (CD133, Notch1, SOX2) when there was no immunostaining or variable weak positivity (<10% of tumor cells).

Statistical analysis. Correlations between p53 protein, p-p53(Ser20), CD133, Notch1, SOX2 expression and clinico-pathological parameters in ovarian carcinomas were statistically studied by the Chi-square test. Associations between p53 protein, p-p53(Ser20), and CD133, Notch1, SOX2 expression were analyzed by the Spearman rank correlation. Associations between the parallel expression of the analyzed proteins and FIGO stages, tumor grade, histological type were performed using the Chi-square test. For all statistical analyses, p53, p-p53(Ser20), CD133, Notch1, SOX2 expression was divided into two groups: negative or limited to 10% of positive tumor cells versus >10–100% of positive cells. Differences were considered significant when $p<0.05$.

Results

Immunoreactivity for p53, p-p53(Ser20) was detected in 40.3%, 44.2% of ovarian carcinomas, respectively. Whereas, p53 and p-p53(Ser20) expression was observed in 70% and 30% of OvBH-1 cells and in 30% and 20% of SW626 cells, respectively. CD133, Notch1, and SOX2 expression was found in 38.4%, 32.6%, and 74.0% of ovarian carcinomas, respectively. Immunoreactivity for CD133, Notch1, and SOX2 was observed in 30%, 20%, and 30% of OvBH-1 cells, respectively. Notch1 and SOX2 expression was observed in 20% and 40% of SW626 cells respectively. CD133 molecule expression was not found in SW626 cells. Variations in p53, p-p53(Ser20) and in CD133, Notch1, SOX2 expressions were observed not only in the whole group of cases, but also in individual ovarian cancer as well as in cell lines. Immunoreactivity for p53 and p-p53(Ser20) was found in >10 to 90% of positive cells. Whereas, expression of CD133, Notch1 and SOX2 was observed in >10 to 60% of positive tumor cells. However, the intensity of immunostaining and range of

positive cells for analyzed biomarkers in ovarian carcinoma tissue was higher in advanced stage and poorly differentiated (Figure 1), than early stage and well differentiated ovarian carcinomas (Figure 2). In OvBH-1 and SW626 cell lines p53, Ser20 and stem cells biomarkers expression showed heterogeneous immunostaining (Figure 3). The association between the analyzed biomarkers and clinicopathological parameters is presented in Table 1. p-p53(Ser20) expression was mainly detected in stages III/IV than in FIGO stage I/II (p=0.003). p53, p-p53(Ser20), Notch1 and SOX2 expression was significantly associated with poorly differentiated (G3) ovarian carcinomas (Table 1). The positive correlation between p53 and p-p53(Ser20) (p=0.02) was found in ovarian carcinomas. However, 42.8% cases with nuclear accumulation of p53 (p53-positive) were negative for p-p53(Ser20) expression, but 35.4% cases without nuclear accumulation of p53

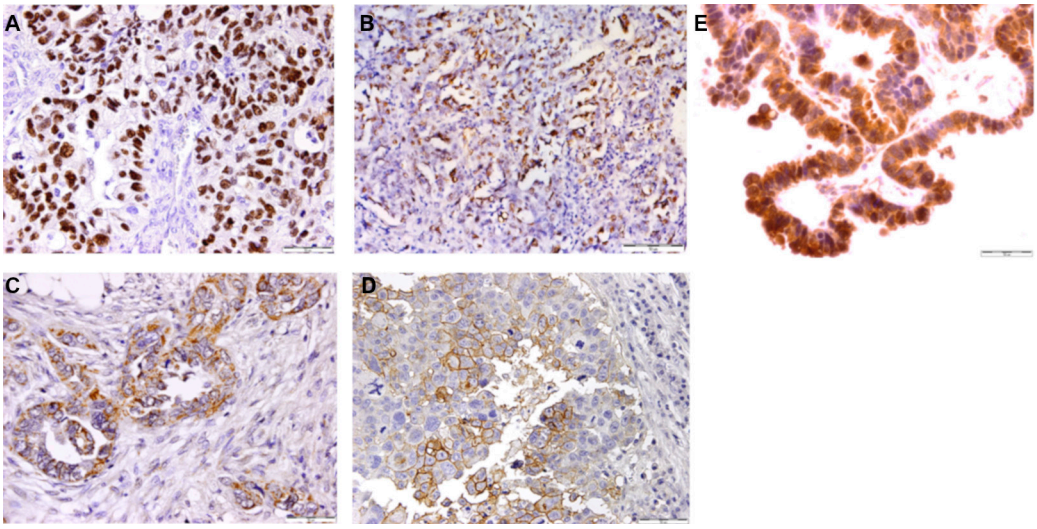

Figure 1. High advanced ovarian carcinomas with p53 protein, p-p53(Ser20), CD133, Notch1, SOX2 expression. Endometrioid ovarian carcinoma FIGO stage III, poorly differentiated (G3) showing strong nuclear accumulation of p53 protein (A), undifferentiated ovarian carcinoma FIGO stage III, expressing p-p53(Ser20) in high percentage of tumor cells (B), serous ovarian carcinoma FIGO III, poorly differentiated (G3) showing cytoplasmic/membrane CD133 expression limited to small area of tumor tissue (C), endometrioid ovarian carcinoma FIGO stage III moderately differentiated (G2), exhibiting heterogeneous membrane Notch1 expression in few tumor cells (D), serous ovarian carcinoma FIGO stage III moderately differentiated (G2), showing extensive cytoplasmic/nuclear SOX2 expression (E) (EnVision technique). The scale bar 50 μm.

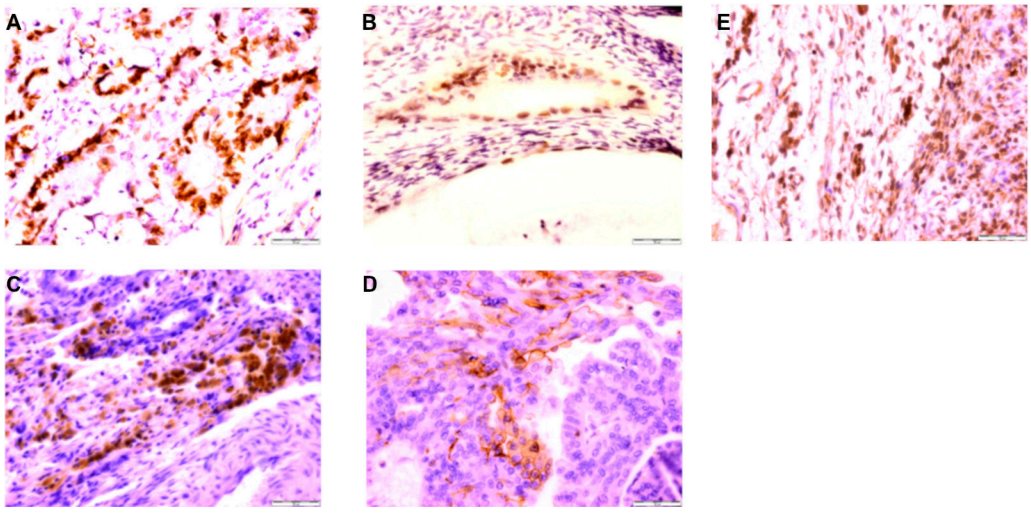

Figure 2. Low advanced ovarian carcinomas with p53 protein, p-p53(Ser20), CD133, Notch1, SOX2 expression. Endometrioid ovarian carcinoma FIGO stage II, well differentiated (G1) heterogeneous pattern of p53 immunoreactivity in tumor tissue (A), mucinous ovarian carcinoma FIGO stage I, well differentiated (G1) weak expression of p-p53(Ser20) limited to a small area of tumor tissue (B), endometrioid ovarian carcinoma FIGO stage II, well differentiated (G1) with focus CD133 expression in tumor tissue (C), serous ovarian carcinoma FIGO stage II, well differentiated (G1) with low expression of Notch1 (D), undifferentiated ovarian carcinoma FIGO stage II heterogeneous pattern of nuclear SOX2 expression is visible (E) (EnVision technique). The scale bar 50μm.

(p53-negative) showed p-p53(Ser20) expression. Association between p53 and SOX2 (p=0.02), p-p53(Ser20) and Notch1 (p=0.03), p-p53(Ser20) and CD133 (p=0.01) expression was observed in ovarian carcinomas. The association between analyzed biomarkers was confirmed by double immunohistochemical staining. Heterogeneous pattern of p-p53(Ser20) and CD133, and p-p53(Ser20) and Notch1 expression was observed in selected cases of ovarian carcinoma. CD133 and Notch1 expression was found in p-p53(Ser20) positive and p-p53(Ser20) negative ovarian carcinoma cells. Co-expression of p-p53(Ser20)/CD133 was observed mainly in p53 positive ovarian carcinoma cells (Figure 4A), whereas p-p53(Ser20)/Notch1 co-expression was limited to single or few carcinoma cells (Figure 4B). In cell lines only OvBH-1 cells showed co-expression of p-p53(Ser20)/CD133 (Figure 4C). Ovarian cancer cells showing parallel expression of CD133, Notch1, SOX2 revealed a more frequent expression of p53 (p=0.002) and p-p53(Ser20) (p=0.0001). In Figure 5, the association between the parallel expression of analyzed biomarkers and clinicopathological parameters of ovarian carcinomas is presented. The expression of p-p53(Ser20)/CD133, p-p53(Ser20)/Notch1, p-p53(Ser20)/SOX2 was observed more often in III/IV than in I/II FIGO stage (p=0.01, p=0.004, p=0.001, respectively) (Figure 5A). p-p53(Ser20)/Notch1, p53/SOX2, p-p53(Ser20)/SOX2 parallel expression was associated with high tumor grade (p=0.01, p=0.005, p=0.009, respectively) (Figure 5B). No correlation between parallel of Ser20 and stem cells biomarkers (CD113, Notch1 SOX2) expression and histological type of ovarian carcinoma was observed. Only p53/SOX2 immunophenotype was found in serous ovarian cancer (p=0.01).

Discussion

Growing evidence demonstrated that p53 loss could lead to an acquisition of stemness in many solid tumors [5, 11, 13]. The role of p53 protein was described in carcinoma stem cells but little is known about p53 protein phosphorylation at serine 20 in CSCs biological behavior [11, 12, 14, 15]. Similarly to earlier published data, we found that p53 nuclear accumulation was associated with worse clinical features of ovarian cancers [20–26]. p53 protein phosphorylation was analyzed very rarely in ovarian carcinomas and there are no data on its expression in ovarian carcinoma stem cells [27, 28]. Observed in current study, expression of p53 protein phosphorylated at Ser20 in 44.2% of cases was associated with advanced stage and poorly differentiated ovarian carcinomas. These results suggest that p53 protein phosphorylation in ovarian cancers might have impact on tumor cells biological, morphological features and enhanced tumor growth [28, 29]. Association between p53 nuclear accumulation and serine 20 expression observed in current paper suggests that p53 protein phosphorylation might concern not only wild type, but also mutant forms of p53 protein overexpressed frequently in advanced and poorly differentiated ovarian carcinomas [22, 25, 26]. This suggestion might be supported by Kaminska et al. [17] results, who found that expression of p53 protein phosphorylated at Ser20 was observed in cell lines of primary ovarian carcinoma and metastasis of a colon adenocarcinoma to ovary with *TP53* gene mutation and high p53 protein accumulation. Also Nguyen et al. [29] indicate that most mutant p53 isoforms

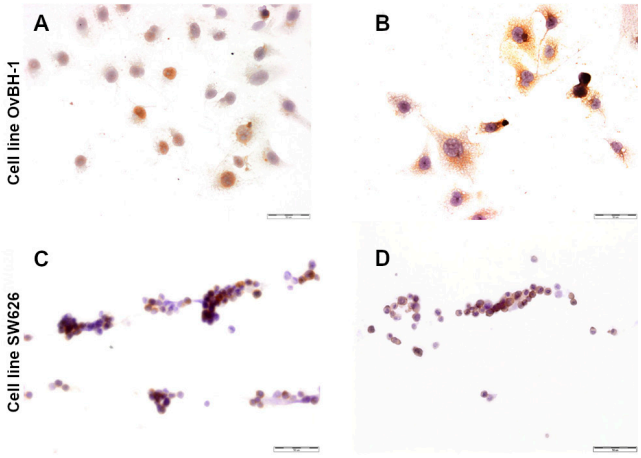

Figure 3. Expression of p-p53(Ser20) and stem cells biomarkers in cell lines: OvBH-1 cell line showed high nuclear p-p53(Ser20) expression (A), limited expression of CD133 molecules in OvBH-1 cells (B), SW626 cells with strong expression of p-p53(Ser20) in high percentage of cells (C), Notch1 expression was detected in large number of SW626 cells (D). (EnVision technique). The scale bar 50μm.

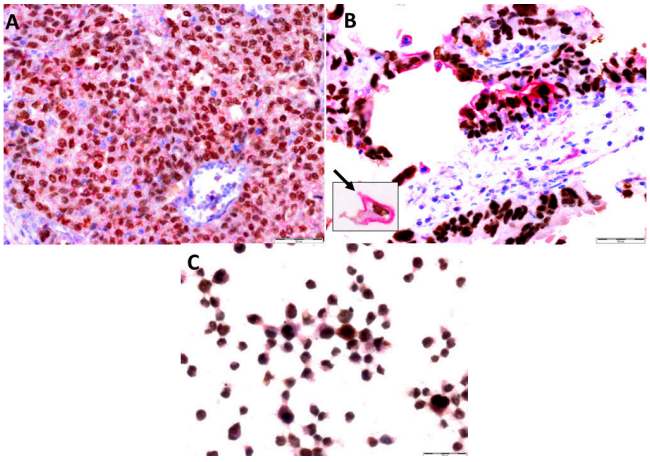

Figure 4. p-p53(Ser20) and CD133, Ser20 and Notch1 double staining in representative cases of ovarian carcinoma and cell line. Serous ovarian carcinoma FIGO stage III, poorly differentiated (G3) with co-expression of p-p53(Ser20) located in the nucleus (brown) and CD133 in the cytoplasm (red immunostaining) observed in moderate percentage of tumor cells (A), endometrioid ovarian carcinoma FIGO stage III, poorly differentiated (G3) showing coexpression of p-p53(Ser20) located in the nucleus (brown) and Notch1 receptor located in the membrane/cytoplasm (red) dominated in malignant tumor cells (marked by arrow) (B). OvBH-1 cell line with coexpression of p-p53(Ser20) visible in nucleus (brown) and CD133 expression visible in the cytoplasm (C). EnVision technique and EnVision DuoFLEX System. The scale bar=50μm.

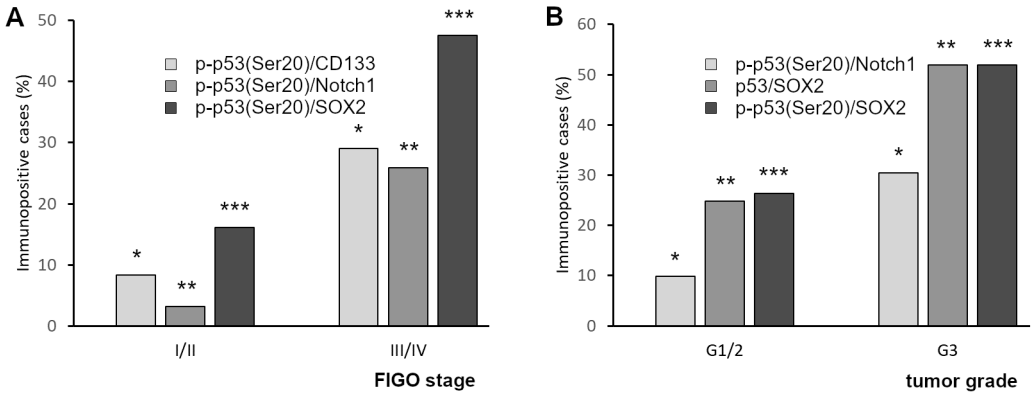

Figure 5. Association between ovarian carcinoma immunophenotype and clinico-pathological parameters. A) Immunophenotypes of p-p53(Ser20)/CD133, p-p53(Ser20)/Notch1, p-p53(Ser20)/SOX2 was dominated in III/IV in comparison to I/II FIGO stage of ovarian carcinomas (*: p=0.01, **: p=0.004, ***: p=0.001); B) Immunophenotypes of p-p53(Ser20)/Notch1, p53/SOX2 p-p53(Ser20)/SOX2 was associated with high ovarian carcinoma grade (*: p=0.01, **: p=0.005, ***: p=0.009).

can be posttranslationaly modified at the same residues as in wild type p53 protein and contribute to a gain of function leading to increased tumor progression. In the literature, there are no data describing Ser20 expression in ovarian carcinoma stem cells. In current study, the stemness features of ovarian carcinoma cells were analyzed by expression of biomarkers occurring on normal adult stem cells e.g. CD133, SOX2, Notch1 [11, 13]. Similarly to published data, the present authors noted that ovarian carcinoma stem cells were identified by CD133 molecules in ovarian tumor tissue [30–33]. However, contrary to the published reports the associations between CD133 expression and clinicopathological parameters of ovarian cancer were not observed in the current study [30, 31]. Nevertheless, there are data suggesting that it is still controversial whether CD133 expression correlated with clinicopathological parameters of ovarian carcinomas and had an impact on the aggressive biological behavior of ovarian cancer [32, 33]. Experimental studies revealed that p53 protein might inhibit CD133 expression and modulate the expression of CSCs specific genes such as: Notch1, SOX2 [5, 12, 13, 26]. The role of p53 phosphorylation was described in normal adult stem cells but in CSCs its role has not been established yet [12, 13]. In the current study, a positive correlation between CD133 and Ser20 expression was observed in ovarian carcinomas. The use of double immunostaining technique allows to reveal the co-expression between p-p53(Ser20) and CD133 molecules in single ovarian carcinoma cell [34, 35]. The co-expression of p-p53(Ser20) and CD133 molecule was found in ovarian carcinomas with nuclear p53 protein expression, but also was observed in cases without p53 protein expression detected by DO-7 antibody. We might consider that p53 protein phosphorylation at serine 20 found in p53-positive and p53-negative ovarian carcinoma cells might concern different forms of p53 protein [12, 25]. The presence of p53 protein phosphorylated at Ser20 in ovarian carcinomas negative for DO-7 antibody immunostaining may point out that the wild type of p53 protein is low, but is stabilized in response to stress inducing marked posttranslational modification of p53 protein [12, 13]. According to previous data, parallel p-p53(Ser20)/CD133 expression accompanied by co-expression of these proteins in p53 negative ovarian cancers indicate that the role of wild-type p53 phosphorylated at Ser20 might be similar to normal adult stem cells and p53 protein phosphorylated at Ser20 can suppress CD133 expression and inhibits tumor growth [11, 12]. Based on published data, we can suggest that wild-type p53 protein phosphorylated at Ser20 might play such roles by suppression of stemness transcription factors [12, 13, 16]. The interactions between p-p53(Ser20) and CD133 expression in p53-positive ovarian carcinomas are not clear now [12, 13, 16]. However, confirmed by double staining the association between parallel expression of p-p53(Ser20)/CD133 and advanced stage of ovarian cancer indicates that ovarian cancer growth might depend not only on stemness features of tumor cells, but also on p53 protein status phosphorylation [17, 28]. Contrary to earlier reports, in the present study Notch1 expression was found in 38.6% of ovarian cancers [6, 36]. Similarly to Wang et al. [32], we found that Notch1 expression was associated with poorly differentiated ovarian cancers. This result indicates that Notch1 expression might be the biomarker characterizing undifferentiated ovarian carcinoma cells and might influence ovarian tumor growth [9, 13, 32]. There are no data on parallel Notch1/p-p53(Ser20) expression in ovarian carcinomas. In current study we found that parallel expression of Notch1/p-p53(Ser20) expression was observed as co-expression of these biomarkers but were visible in small number of ovarian carcinoma cells. These results indicate that Notch1 expression might depend on p-p53(Ser20) expression and the role of Notch1 in ovarian carcinomas might be determined by p53 protein phosphorylation. We postulated that co-expression of Notch1/p-p53(Ser20) might characterize

subclones of ovarian carcinoma cells, which possess features promoting their selective growth in a tumor mass. There are strong evidences showing correlation between *TP53* gene and Notch members in ovarian cancer, which are involved in various aspects of stem cells maintenance, cell differentiation and tumor progression [26]. We found that parallel Notch1/p-p53(Ser20) expression was accompanied by co-expression of Notch1/p-p53(Ser20) and was correlated with advanced and poorly differentiated ovarian cancers. This observation might be partly compared with Chen et al. [26], who revealed that *TP53* status significantly impact the prognostic value of Notch 1 expression in ovarian cancer. The role of SOX2 expression as an important marker, which demonstrates an oncogenic function in epithelial cancer cells by promoting cell proliferation, cell migration and tumorigenic potential was found [6, 13, 33]. In the present study SOX2 expression was observed in high percentage of poorly differentiated ovarian carcinomas [7, 37]. This observation suggests that the activity of SOX2 in poorly differentiated ovarian carcinomas might promote their malignancy and tumor growth [7, 11, 13, 38, 39]. In the current study, an observed association between parallel p53 nuclear accumulation and SOX2 expression in poorly differentiated ovarian carcinomas is in agreement with published studies. These indicate that nuclear accumulation of p53 protein might enhance transcriptional activity of SOX2 in tumor cells and facilitate their function as an oncogene promoting stemness phenotype of ovarian cancer reflecting the biologically aggressive feature of ovarian cancer [7, 11, 13, 15, 38, 39]. Up to now there are no data on association between p-p53(Ser20) and SOX2 expression in ovarian carcinomas. In the next step of our research, we analyzed the association between p53 phosphorylation at Ser20 and SOX2 expression in ovarian carcinomas. We found that in the whole group of analyzed cases the correlation between p-p53(Ser20) and SOX2 was not observed. However, parallel expression of both SOX2 and p-p53(Ser20) was associated with advanced stage and poorly differentiated ovarian cancers. According to published results, we might conclude that regulation of SOX2 transcriptional activity might depend on p53 protein phosphorylated form at serine 20 and has an impact on ovarian carcinoma cells behavior [11–13]. This suggestion might be supported by data, which revealed that wild-type p53 loss has been associated with enhancing the efficiency of somatic cell reprogramming to a pluripotent state and stem-like phenotype in cancer [11, 13, 39]. Revealed in the current paper, association between the parallel expression of p-p53(Ser20)/Notch1, p-p53(Ser20)/CD133 accompanied by co-expression of these biomarkers and advanced and poorly differentiated ovarian carcinomas indicate that p53 protein phosphorylation at serine 20 might concern different forms of p53 protein expressed by ovarian cancer stem cells and might implicate CSCs motility and invasion leading to different biological behavior of ovarian cancer [11, 13, 39]. Found in OvBH-1 cell line with *TP53* gene mutation co-expression between p-p53(Ser20) and CD133 might confirm our hypothesis. On the other hand, some authors suggested that the role of p53 protein phosphorylation as a pivotal mechanism, which controls expression of transcriptional factors in adult stem cells and prevents uncontrolled de-differentiation of stem cells [11–14]. According to published results, in our opinion this mechanism might be disturbed due to the phosphorylation of different forms of p53 protein [12–14]. Results from this study showed that correlation between p-p53(Ser20) and CD133, Notch1, expression reflects co-expression of these proteins in single ovarian carcinoma cell indicating that p-p53(Ser20) might play stemness role in ovarian carcinomas and cooperation between these markers might have impact on biological behavior of ovarian cancer. Association between p53 phosphorylated at serine 20 and carcinoma stem cells biomarkers suggest that these biomarkers cooperate and network between these proteins might facilitate ovarian tumor growth. Association between parallel p-p53(Ser20)/Notch1, p-p53(Ser20)/CD133, p-p53(Ser20)/SOX2 expression and advanced stage and high tumor grade indicate that these biomarkers enhancement malignancy of ovarian carcinomas with stem cell phenotype.

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