

# TGF- $\beta$ expression in vulvar cancer

## Ekspresja TGF- $\beta$ w raku sromu

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### Abstract

*Vulvar cancer accounts for about 3-5% of all female genital carcinomas. TGF- $\beta$  protein is a member of a superfamily of cytokines that regulate cell functions. A correlation between this protein and many neoplastic processes was reported.*

**Objectives:** *In our study we analyzed TGF- $\beta$  expression in vulvar tumor among patients with diagnosed squamous cell carcinoma (with and without inguinal nodes metastases).*

**Material and methods:** *Paraffin embedded blocks obtained from vulvar tissues and inguinal nodes (from 31 patients with vulvar carcinoma FIGO II-IV) were prepared. Next, the hematoxylin and eosin staining was performed. Monoclonal antibody NCL-TGF- $\beta$  was used for immunohistochemical tests.*

**Results:** *Higher expression of TGF- $\beta$  in cancer cells corresponds to more advanced cancer stages (FIGO). A positive correlation between TGF- $\beta$  and metastases, as well as a number of inguinal nodes metastases was observed. The ratio between the number of stained cells in vulvar tumor and of inflammatory cells proved to be higher in FIGO stage III than IV. Possibly, TGF- $\beta$  increase in vulvar tumor contributes to the breakdown of immunological processes limiting cancer progression. Higher TGF- $\beta$  expression leads to metastasis in regional lymphatic nodes.*

**Conclusions:** *TGF- $\beta$  overproduction is observed in vulvar neoplastic processes. In early stages of carcinogenesis TGF- $\beta$  inhibits cancer cell proliferation, but in more advanced stages it accelerates cancer progression by inhibiting the immunological response.*

Key words: **vulva / cancer / factor beta (TGF- $\beta$ ) /**

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## Streszczenie

Rak sromu stanowi 3-5% wszystkich nowotworów narządu płciowego kobiety. TGF- $\beta$  jest białkiem z rodziny cytokin biorących udział w regulowaniu cyklu komórkowego. W licznych pracach wykazano związek tego białka z procesem nowotworowym.

**Cel pracy:** W badaniu przeprowadzono ocenę ekspresji TGF- $\beta$  w tkankach guza sromu w grupie pacjentek z potwierdzonym histopatologicznie rakiem płaskonabłonkowym (z obecnymi lub brakiem przerzutów do węzłów chłonnych).

**Materiał i metody:** Przygotowano bloczki parafinowe z tkanek guza sromu oraz węzłów chłonnych (w grupie 31 pacjentek z rakiem sromu FIGO II-IV). Następnie, po zastosowaniu barwienia hematoksyliny-eozyna, przeprowadzono badanie przy użyciu monoklonarnego przeciwciała NCL-TGF- $\beta$ .

**Wyniki:** Zaobserwowano, że wraz z zaawansowaniem procesu nowotworowego (wg skali FIGO) wzrasta ekspresja TGF- $\beta$ . Istnieje dodatnia korelacja pomiędzy TGF- $\beta$  a obecnością przerzutów w węzłach chłonnych pachwinowych. Wykazano, że stosunek liczby wybarwionych komórek w tkance guza sromu do liczby komórek zapalnych jest większy w stopniu III niż w stopniu IV. Prawdopodobnie wzrost TGF- $\beta$  przyczynia się do załamania procesów immunologicznych ograniczających progresję choroby nowotworowej. Wyższa ekspresja TGF- $\beta$  prowadzi do rozwoju przerzutów w regionalnych węzłach chłonnych.

**Wnioski:** W przypadkach nowotworu sromu obserwuje się zwiększoną produkcję TGF- $\beta$ . We wczesnych stadiach choroby nowotworowej TGF- $\beta$  wpływa hamująco na proliferację komórek nowotworowych, natomiast w stadiach bardziej zaawansowanych przyspiesza postęp choroby poprzez hamowanie odpowiedzi immunologicznej.

Słowa kluczowe: srom / rak / TGF- $\beta$  /

## Introduction

Vulvar cancer accounts for about 3-5% of all female genital carcinomas. About 95% of invasive cancers belong to the squamous type. Vulvar cancer is more often diagnosed in women with diabetes, obesity and arterial hypertension [1, 2]. Additional risk factors include age and lifestyle (cigarette smoking, socio-cultural level, hygienic habits and sexual behaviors) [3, 4, 5, 6]. The oncogenic impact of viral infections has also been reported (HSV2, HPV) [7, 8, 9]. The lymphatic rete of the vulva is well developed, thus this route is particularly preferential for cancer cells to spread. The first level of carcinoma metastases is the level of the inguinal lymphatic nodes.

Cytokines, among them TGF- $\beta$  protein, regulate cell functions (inter alia activity of immunized cells), modify hematopoiesis, as well as participate in processes of cell proliferation and/or differentiation. Platelets, macrophages, neutrophils and lymphocytes are the main sources of TGF- $\beta$  [10, 11, 12]. Decorin and biglycan are known TGF- $\beta$  inhibitors [13, 14, 15, 16]. TGF- $\beta$  inhibitory activity is manifested in cell cycle blocking (in G1/S phase, G phase and G0/G1 phase) [17, 18]. TGF- $\beta$  is a potential proliferative inhibitor of epithelial, endothelial cells and hepatocytes, lymphocytes and hematopoietic cells [19,20,21,22]. TGF- $\beta$  is able to induce apoptosis in the above mentioned cells, as well as in some nervous system cells [23,24,25]. TGF- $\beta$  induces mesenchymal cell proliferation and increases ECM protein production [26, 27]. This protein is able to decrease or increase angiogenesis (through VEGF secretion, MMP2/9 activity) [28, 29]. It supports the production of substances that prevent ECM degradation and it stimulates EMT (epithelial-mesenchymal cell transition), which plays a key role in neoplastic invasion and metastases [30,31].

TGF- $\beta$  is an important mediator in the process of inflammation and wound healing [32]. It influences chemotaxis of monocytes,

lymphocytes and neutrophils [33,34] and is considered a strong immunosuppressive modifier of cell-mediated response and humoral response by affecting the CD4+/CD8+ lymphocytes [35, 36].

Neoplastic cells are susceptible to TGF- $\beta$  inhibitory role in the early stages of disease. With cancer progression, these cells lose their susceptibility and become autonomous to TGF- $\beta$ . This relation is probably caused by TGF- $\beta$  receptor mutation (TGF- $\beta$  RI and TGF- $\beta$  RII) that makes neoplasm cells resistant to the inhibitory effect of TGF- $\beta$  [37, 38]. A well-documented proangiogenic and immunosuppressive activity of TGF- $\beta$  favors the growth of neoplastic tumor, increases cancer invasiveness and induces metastasis [39]. The connection between TGF- $\beta$  and its isoforms with hepar sclerosis, hepatocirrhosis and hepatocellular cancer is emphasized [38]. There exists a link between TGF- $\beta$  titer and node metastases in breast cancer [40,41]. A correlation between this protein and cervical, ovarian, endometrial, esophageal, laryngeal, colon, pancreatic, pulmonary, and gastric cancers was reported. [42, 43, 44, 45, 46, 47, 48, 49, 50, 51].

Apart from the proangiogenic and proinflammatory activity of TGF- $\beta$ , a relationship between its titer and cancer endovascular invasiveness and the presence of metastases, particularly in lymphatic nodes and liver, was demonstrated [52,53]. In case of head and neck squamous cell carcinomas, as well as in oral and cervical cancers, a positive correlation between high TGF- $\beta$  titer and cancer staging, metastases and unfavorable prognosis was observed [53, 54, 55].

Li AG et al., described the role of TGF- $\beta$  in the neoplastic processes in the skin [56]. Data on the role of TGF- $\beta$  in vulvar cancer are scarce. The available literature neither supports the theory about the applicability of TGF- $\beta$  as a marker of cancer staging, nor confirms the relation between TGF- $\beta$  and lymph nodes metastases.

## Objectives

The aim of the study was to analyze TGF-β expression in vulvar tumors among 31 patients with squamous cell carcinoma (with and without inguinal nodes metastases).

## Material and methods

Paraffin-embedded vulvar tissue and inguinal node blocks were obtained from 31 patients with FIGO II-IV vulvar carcinoma (operated on at the Clinic of Gynecology, Obstetrics and Oncological Gynecology, Medical University of Silesia, Poland, with the use of the Way or Hacker technique). Then the hematoxylin and eosin staining was performed. NCL-TGF-β (Novocastra, S3001) monoclonal antibody (1:100 dilution) was used for the immunohistochemical tests. The slides were prepared with the use of Strept ABC complex/ARP Duet mouse/rabbit product (DAKO, K0492). DAB (DAKO, S3466) was the chromogen used for staining. All immunohistochemical reactions followed the producer's instructions.

We investigated slides with vulvar tumor and inguinal nodes metastases. In case of patients without metastases, TGF-β was identified only in the vulvar tumor.

The following were examined in tumor tissue:

1. Staining intensity in cytoplasmic granules of the neoplastic cells (staining intensity in basal cells of normal epidermis was used as a reference point). Scale: “-“ no staining reaction, “+” weak staining reaction, “++” moderate staining reaction, “+++” strong staining reaction.
2. Type of neoplastic invasion (microfocal, macrofocal, mixed and dispersed)
3. Type of inflammatory infiltration in the environment surrounding the tumor (weak, moderate, strong).
4. Number of neoplastic cells with cytoplasmic granules stained with anti TGF-β antibody per 1000 neoplastic cells.
5. The ratio between the number of neoplastic cells stained with anti TGF-β antibody and the number of inflammatory cells in 5 visual fields.

The following were examined in the inguinal nodes metastases:

1. Staining intensity in cytoplasmic granules of the neoplastic cells (methodology as described above).
2. Number of neoplastic cells with cytoplasmic granules with anti TGF-β antibody per 1000 neoplastic cells.
3. Type of neoplastic invasion.

## Results

Results of the examined tumor tissues:

1. Out of 31 cases, 3 showed no staining reaction in cytoplasmic granules of the neoplastic cells, 22 - weak staining reaction, 5 - moderate staining reaction and 1 - strong staining reaction.
2. Microfocal, macrofocal, and mixed invasions were found in 14, 9, and 6 cases, respectively. In 2 cases we observed dispersed invasion.
3. We found 13 weak, 10 medium and 8 strong inflammatory infiltrations in tumor tissues.

4. Among 1000 neoplastic cells, 281 were stained with anti TGF-β antibody, what makes 28% of all neoplastic cells in tumor tissues.
5. The ratio between the number of stained cells and the number of inflammatory cells in 5 visual fields was 5.64, and was the highest in patients with FIGO III.

Results of the examined inguinal nodes metastases:

1. Out of 17 cases with inguinal nodes metastases, no staining reaction in cytoplasmic granules of the neoplastic cells was observed in 1, weak staining in 11, moderate in 4 and strong in 1 case.
2. Among 1.000 neoplastic cells in the inguinal nodes, 262 were stained with anti TGF-β antibody, what amounts to 26% of all neoplastic cells in the metastatic tissues.
3. Microfocal invasion was the most common type of neoplastic invasion.

Our analysis showed that the increase of stained neoplastic cells in vulvar tumor is accompanied by an increase of stained neoplastic cells in the inguinal nodes. The number of TGF-β antibody stained cells in vulvar tissues rises with the FIGO stage. However, such correlation was not observed in case of inguinal nodes metastases.

The ratio between the number of stained cells in vulvar tumor and the number of inflammatory cells was 8.55, 3.65 and 1.80 in patients with FIGO stages III, IV and II, respectively. In cases when inguinal nodes metastases were present, we noticed that an increasing number of stained neoplastic cells in the metastatic tissues was accompanied by a decreased intensity of staining for the inflammatory tissue surrounding the tumor.

Microfocal invasion was the most common type in vulvar tumor and in metastatic inguinal nodes. The highest ratio of TGF-β antibody stained neoplastic cells was observed in 2 cases of dispersed invasion of the vulvar tumor. No dispersed invasion was found in metastatic inguinal nodes.

## Discussion

The issue of chronic inflammation in vulvar cancer pathogenesis has been tackled in many papers [25, 57]. The role of infectious factors such as viruses (HPV), bacteria, Chlamydia and proinflammatory cytokines: VEGF, TGF-α, is emphasized. In our research we attempted to analyze the influence of inflammatory processes on the neoplastic progress. In light of our results, we conclude that patients with strong inflammatory processes in vulvar tumor suffered from less advanced vulvar cancer. Only 25% of that group had inguinal nodes metastases. In comparison, 50% of patients with moderate inflammatory staining had inguinal nodes metastases. Possibly, the process of inflammation in tumor tissues, in other words a breach in the immunological barrier, results in acceleration of the neoplastic process. It also facilitates development of metastases in the regional nodes. Our data showed that intensity of inflammatory infiltration in tumor tissues decreases with neoplastic progress.

It seems that microfocal invasion is more often associated with the presence of regional nodes metastases and unfavorable prognosis for the patient. Drew et al., found dispersed invasion, which we observed in 2 cases, to be the sign of the least favorable prognosis for the patient [58].

Cancer cells have been known to produce TGF-β in an autonomous way. In physiological processes it plays the role of cell cycle inhibitor and immunosuppressive agent. Simultaneously, TGF-β increases angiogenesis and extracellular matrix synthesis. Paradoxically, TGF-β overproduction is observed in neoplastic processes. TGF-β inhibits cancer cell proliferation in early stages of carcinogenesis but in more advanced stages it accelerates cancer progression [54]. In the literature this contradiction is explained in a number of ways. Firstly, cancer cells produce TGF-β in an autonomous way, leading to its overexpression in vulvar tumor [59]. Secondly, cancer cells lose their TGF-β receptors, what makes them independent of their inhibitory influence [60]. In our study we demonstrated that the number of cancer cells stained with TGF-β antibody in vulvar cancer increases with FIGO stages. However, we did not notice such a correlation in the inguinal nodes metastases.

The relationship between intensity of TGF-β expression in vulvar cancer and the risk of inguinal nodes metastases is emphasized [61]. TGF-β expression was shown in about 90% of the slides from vulvar tumors and in about 88% of the slides from inguinal nodes metastases. Our analysis indicated that the increase of stained cells in vulvar tumor is accompanied by the increase of TGF-β stained cells in lymphatic nodes tissues.

Studies of Logullo AF et al., Natsugoe S et al., and Bristow RE et al., confirm that TGF-β expression depends on tumor location and its biology [61,62,63].

The ratio between the number of stained cells in vulvar tumor and to the number of inflammatory cells proved to be higher in FIGO stage III than IV. We are of the opinion that it may be caused by more extensive necrosis in FIGO stage IV than III. Positive correlation between TGF-β and the presence of metastases and the number of inguinal nodes metastases was observed. High expression of TGF-β can be assumed to inhibit the immunological response, contributing to cancer progression and the development of the inguinal nodes metastases.

## Conclusion

TGF-β expression in cancer cells increased with more advanced cancer processes (FIGO), what (in immunohistochemical test of vulvar tumor) is manifested by an increasing ratio between the number of stained cells in vulvar tumor and of inflammatory cells, as well as by a higher number of stained cells per 1.000 cancer cells. Supposedly, TGF-β increase in vulvar tumor contributes to the breakdown of the immunological processes limiting cancer progression. Higher TGF-β expression leads to metastasis in the regional lymphatic nodes.

Our research requires further analysis. It should be emphasized that tumor histopathology and tumor clinical manifestation depend on its environment. Probably, future studies concerning TGF-β in neoplastic processes, as well as in vulvar cancer, should concentrate on TGF-β isoforms and their receptors.

## Oświadczenie autorów:

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Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związanego z powstawaniem pracy.

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