Vulvar cancer as a target for molecular medicine

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1. ABSTRACT

Vulvar carcinoma is a rare female genital neoplasm. Although numerous molecular defects in vulvar carcinomas have been reported until now, no molecular markers that could be applied in daily clinical work have been identified so far. However, there is emerging evidence that specific mutations and gene expression patterns may be used as diagnostic tools in oncology. In this article we systematically review genetic alterations that may contribute to the development and progression of vulvar carcinoma. We conclude by suggesting molecular markers of potential clinical value in the diagnostics of this type of cancer.

2. INTRODUCTION

Cancer is the second most common cause of death in modern society. An increased risk of tumor formation is one of the consequences of gradually extending length of human life. Despite numerous improvements in cancer management, the analysis of mortality trends predicts an 11% increase in deaths caused by cancer in the countries of the European Union in 2015 (in comparison to the year 2000) (1). One potential way of dealing with increased risk of cancer is the improvement of early diagnostic tools, especially the greater use of molecular markers of increased genetic risk for vulvar cancer.
Endometrial biomarker

Table 1. General features of two basic subtypes of VSCC

<table>
<thead>
<tr>
<th>Feature</th>
<th>HPV-related</th>
<th>HPV-unrelated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of diagnosis</td>
<td>Younger</td>
<td>Elderly</td>
</tr>
<tr>
<td>Subtype</td>
<td>Basaloid verrucous</td>
<td>(non-keratinizing)</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Better</td>
<td>Worse</td>
</tr>
</tbody>
</table>

Table 2. VIN subtypes and its characteristics with regard to vulvar cancer development (8)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Usual VIN (uVIN)</th>
<th>Differentiated (dVIN)</th>
<th>VIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence/incidence</td>
<td>Common</td>
<td>Less common</td>
<td></td>
</tr>
<tr>
<td>Age of patients</td>
<td>Younger</td>
<td>Elderly</td>
<td></td>
</tr>
<tr>
<td>HPV status</td>
<td>Positive (especially HPV 16)</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Usual lesions</td>
<td>Multifocal</td>
<td>Unifocal</td>
<td></td>
</tr>
<tr>
<td>Possibility of progression to SCC</td>
<td>3-4%</td>
<td>Rapid progression- of ten</td>
<td></td>
</tr>
<tr>
<td>Subtype of VSCC developed</td>
<td>Warty, Basaloid</td>
<td>Keratinizing/non keratinizing</td>
<td></td>
</tr>
<tr>
<td>Other:</td>
<td>More frequent among VC patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular characteristics (9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV (DNA)</td>
<td>Positive</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>p16INK4</td>
<td>positive</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>MIB1</td>
<td>positive</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td>negative</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>uVIN-SCC-53 y.o.</td>
<td>uVIN-SCC-36 y.o.</td>
<td>dVIN-SCC-not present</td>
</tr>
</tbody>
</table>

2.1. Epidemiology

Most commonly, this type of cancer is diagnosed among older women. The average age at diagnosis is 67.5 years, while the highest number of patients is diagnosed in their seventies. The highest rate of death due to vulvar cancer occurs for women aged seventy and more (2). Furthermore, the incidence of vulvar cancer has been on the increase over the past few decades. Also, over the past 20 years an alarming trend among young women has been observed: the increase of both VSCC (Vulvar Squamous Cell Carcinoma) and pre-neoplastic lesions occurrence may be related to the increase in prevalence of HPV (human papillomavirus) infections (3).

The vulvar carcinoma is rare female genital neoplasm. According to the American Cancer Society (ACS), vulvar cancer is responsible for 4% of cancers of the female reproductive organs and 0.6% of all cancers among American women. Their risk to develop vulvar cancer at some point during lifetime is 1 in 406. The ACS’s most recent estimates (2009) suggest that about 3,580 cases are diagnosed and circa 900 women will die because of it in a given year (2). In comparison in Poland (the authors’ homeland) this cancer accounts for 2.5 % of all cancer cases among women, and contributes to over 5% of all gynecological cancers, which places it in the 4th position for morbidity frequency - after breast, cervix and endometrial carcinomas (1). On average, approximately 400 cases are diagnosed in Poland every year and about 50% of those patients die as a result of the disease.

2.2. Cancer types

According to histopathological criteria four main types of vulvar cancer are diagnosed: squamous cell carcinoma (SCC), granular cell tumor, adenocarcinoma and melanoma. Squamous cell carcinoma accounts for approximately 95% of all vulvar cancer cases. Among this type the following subtypes have been identified: keratinizing /non-keratinizing squamous cell carcinoma (KSC/NKSC), basaloid squamous carcinoma (BC), warty squamous cell carcinoma (WC) and verrucous squamous cell carcinoma (4). Furthermore, two different etiological sub-types of SCC have been suggested. The first one - linked to HPV infection (and smoking), is usually preceded by vulvar intraepithelial neoplasia (VIN) and diagnosed in younger patients. The second type, more frequently diagnosed and characteristic for older patients is not related to HPV and commonly arises from vulvar dystrophic lesions (5) (see Table 1).

2.3. Precancerous stages

2.3.1. Vulvar intraepithelial neoplasia (VIN)

A pre-neoplastic stage of the vulva is VIN (Vulvar Intraepithelial Neoplasia). Initially, according to the International Society for the Study of Vulvar Disease (ISSVD) guidelines, VIN has been divided into 3 grades, depending on the degree to which epithelial boundaries are disrupted, with VIN III identified as carcinoma in situ (6). The risk of VIN progression into the invasive form is estimated at 3-8%, and the highest risk has been observed for lesions of non-HPV origin (7). Recently, a modified terminology based on the morphologic criteria, has been proposed (8). Typical VIN consists of lesion previously defined as VIN II and III lesions of basaloid and warranty type, while a previously differentiated VIN III is now recognized as differentiated VIN. Molecular analysis seems to supports new diagnostic criteria (9). It is molecular biology that gives an additional evidence that these two distinct types of VIN are actually precursors of cancers with different clinical characteristic and outcome (see Table 2).

2.3.2. Lichen sclerosus (LS)

Another condition associated with vulvar cancer development is lichen sclerosus (LS) that is commonly found in patients with keratinizing subtype of the disease. It is a prevalent inflammatory dermatosis, affecting vulvar and perianal areas. Although it seems to be related to autoimmune disorders, its causes remain unknown (10).

3. MOLECULAR BIOLOGY OF HUMAN VULVAR CANCER

3.1. Two pathways of vulvar carcinogenesis

At least two different pathways of vulvar carcinogenesis have been proposed: 1) HPV-dependent model and 2) HPV-independent model (11-14). The presence of HPV infection, especially of high-risk type viruses (including HPV 16, HPV 18, HPV 31), corresponds with the development of warty or basaloid vulvar intraepithelial neoplasia. At the same time numerous interesting genetic alterations were reported in HPV-negative vulvar cancer (15). It is generally difficult to critically review vulvar cancer molecular biology due to limited data available in the field as well as due to missing pathological reports on patients’ HPV status. In the following section we review the role of HPV infection, the main genetic pathways leading to carcinoma, as well as other selected alterations present in VSCC, focusing on correlations between genetic alterations and clinical data, where available.
3.2. HPV infection as main vulvar transformation factor

HPV infection has been proven to be the main etiological factor of cervical carcinoma development (16). It has also been associated with the development of vulvar cancer (5). According to statistical analysis, HPV is detectable in 40.1% of vulvar cancer cases, 80.4% of VIN II/III and in 77.5% of VIN I (11). Moreover, most of HPV-positive tumors carry subtype 16 of the virus (15). HPV is mainly found in basaloid and warty subtype of the disease and rarely in keratinizing or nonkeratinizing subtypes (14, 17, 18).

The role of HPV in cancer development and progression is dependent on the carcinogenic function of viral proteins E6 and E7 (19). These oncoproteins interact with several cellular targets – including p53 and pRb - altering their functions and subsequently distorting the cell-cycle (20-22). In particular, E6 blocks apoptosis through ubiquitin-mediated degradation of p53 and by preventing the translocation of p53 from the cytoplasm into the nucleus. At the same time, E6 alters the transcription of multiple genes including c-myc, cytokines and immune signaling proteins. Transcription is blocked possibly due to its interaction with p300/CBP (23). Moreover, E6 is also able to increase cellular lifespan by increasing telomerase activity (24). On the other hand, E7 binds to hypophosphorylated Rb protein (active) and subsequently disrupts its interaction with E2F, prematurely promoting entry into S-phase (25, 26). Other targets of E7 include histone deacetylases, AP-1 and transcription factors, cyclin-dependent kinases and cdk inhibitors (21, 22).

HPV infection is followed by viral genome integration into the host cell genetic material. The analysis of over 190 integration sites found in tumors, tumor-derived cell lines and dysplastic lesions, revealed that integration events occur in the entire host genome. Sequences disrupted by HPV-insertional mutagenesis include genes involved in cancer formation like MYC or hTERT (27). HPV status was also shown to correlate with chromosomal aberrations, especially in 3q (22-25) or 8q (21) and 11q (28). On the other hand, HPV-negative vulvar cancers were shown to harbor TP53 gene mutations (29-31) and allelic imbalance at 17p13.1 locus (32). However, till now no specific pattern of chromosomal rearrangements has been described.

3.3. Cell-cycle alterations in vulvar carcinogenesis

Molecular study of two different subtypes of vulvar premalignant lesions (characteristic for distinct types of tumor development) showed that apart from HPV status, they can be distinguished by the levels of gene expression of p53, p16INKnk4a and MIB-1 (9). The first two (p53, p16INKnk4a) are proteins involved in important signaling pathways (12, 33). The key molecules of those pathway are also p14ARF, MDM2, cyclin D1, cyclin-dependent kinases 4/6 and pRb (34). To highlight the mechanisms responsible for VSCC pathogenesis and to indicate the molecular markers of VSCC, all data reported on those proteins and their interactors have been analyzed and conclusions of these studies in the context of their clinical relevance are briefly summarized below (see Table 3 and 4).

3.4. TP53 as a vulvar cancer guardian

TP53 gene alterations were found in multiple studies carried on VSCC samples (17, 29, 35). TP53 point mutations were reported mostly in HPV-negative tumors (17, 29), albeit the presence of the virus genome did not affect preferential LOH at the TP53 locus (29). Pinto et al. (36) have recently reported that two of four TP53-positive VSCCs shared identical gene alterations within adjacent differentiated vulvar intraepithelial neoplasia. Interestingly, the overexpression of p53 was also frequently reported in vulvar cancer patients (30, 31, 37). However, studies combining both the immunohistochemical and molecular techniques, have revealed that the overexpression of p53 does not necessarily correspond to gene mutations in VSCC (17).

Multiple interactors of p53 seem to be involved in the process of vulvar cancer development (38, 39). Specific importance must be attributed to the p53-E6 interaction (34, 40). Although HPV E6 protein binds to TP53 and promotes its rapid degradation, the overexpression of this protein does not necessarily correspond to HPV status in patients affected by VSCC (30) (see Table 3 and 4). Although some reports show that TP53 gene point mutations correlate with poor survival of VSCC patients (35), other do not support this observation (29). Moreover, p53 overexpression was suggested to be a late event in VSCC development, co-occurring with lymph node metastasis (31, 37). However, not many clinical tests and/or molecular markers may be used in the diagnostics of precancerous/cancerous vulvar lesions on the basis of the currently available data. In particular, neither the overexpression of p53 nor HVP infection can be associated with disease-free survival of women suffering from VSCC (30). Summary of all reports on TP53 and/or p53 in vulvar cancer does not give clear picture of its role in vulvar cancer development. Therefore more studies on large cohorts of patients are essential to clarify the disturbances of p53 pathway in vulvar cancer.

3.5. p21WAF/CIP as a marker of vulvar cancer metastases

The p21 / WAF1, known as cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1, encoded by the CDKN1A gene, is main downstream target of p53 pathway. p21WAF/CIP inhibiting cyclin dependent kinases (in response to TP53-mediated damage) and blocking DNA replication (PCNA) is expected to abrogate cell-cycle mechanisms. Those functions were shown to be blocked by interaction with HPV type 16 E7 oncoprotein (41). Moreover, the WAF/CIP gene is involved in apoptosis inhibition, thus may act either as a tumor suppressor or as a protooncogene (42). To our knowledge, until today p21WAF/CIP gene mutational status has not been assessed in VSCC, but low expression of this protein was observed in 70% of well-differentiated vulvar carcinomas (43). On the contrary, high expression of p21WAF/CIP was significantly correlated with decreased disease-free survival of patients staged I/II (43). In the study of advanced VSCC cases, increased p21WAF/CIP expression was reported in lymph node metastases, suggesting a metastasis moderator role of this protein (44). Recently, however, a review of molecular
Endometrial biomarker

Table 3. Genetic and epigenetic alterations in VSCC and cancer associated lesions

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>Histological type/lesion</th>
<th>FIGO stage</th>
<th>HPV status</th>
<th>TP53 status</th>
<th>Survival/prognosis</th>
<th>Remarks</th>
<th>No</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TP53</td>
<td>Mutation</td>
<td>VSCC/SLS</td>
<td>ND</td>
<td>- / -</td>
<td>77.8% of VSCCs, 46.7% of LS, 22.2% of SH</td>
<td>NI Mutation in non neoplastic lesions may lead to VSCC development</td>
<td>27</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>TP53</td>
<td>LOH</td>
<td>VSCC</td>
<td>LIV</td>
<td>+ / 16</td>
<td>23/36; *LOH, more frequent in case of 72R P that in 72R</td>
<td>NI LOH irrespective of HPV status</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>TP53</td>
<td>Mutation</td>
<td>VSCC</td>
<td>LIV</td>
<td>- / 16</td>
<td>23/36; *LOH, more frequent in case of 72R P that in 72R</td>
<td>NI TP53 mutation more common in HPV negative</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>TP53</td>
<td>Mutation</td>
<td>VSCC</td>
<td>LIV</td>
<td>- / -</td>
<td>12/38 More common in advanced stages (III IV)</td>
<td>*correlation with survival/overall survival</td>
<td>38</td>
<td>(35)</td>
</tr>
<tr>
<td>5</td>
<td>TP53</td>
<td>Mutation</td>
<td>VSCC K,NK, W,B</td>
<td>ND</td>
<td>+ / 13</td>
<td>13/31; *correlation with survival</td>
<td>NI 40% in HPV +, 68.8% in HPV - HPV not in K and NK</td>
<td>21</td>
<td>(17)</td>
</tr>
<tr>
<td>6</td>
<td>14-3-3 sigma</td>
<td>Methylation</td>
<td>VSCC VIN</td>
<td>ND</td>
<td>+ / 16</td>
<td>66% of VSCC; Present in late VIN</td>
<td>NI Metylation of 14-3-3 associated with methylation of p16Ink4a/No mutations</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>p16ink4a</td>
<td>Methylation</td>
<td>VSSC VIN</td>
<td>ND</td>
<td>- / 16</td>
<td>13/36 of VSCC; Present in late VIN</td>
<td>NI Metylation of p16Ink4a coexists with methylation of 14-3-3/ Possibly early event in VC development/ Irreproducible of p53 status and HPV</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>p16ink4a</td>
<td>Metylation</td>
<td>VSCC VIN,LS</td>
<td>LIV</td>
<td>- / -</td>
<td>68% of VC, 69.2% of VIN, 42.8% of LS</td>
<td>NI Possibly an early event in transformation, but insufficient by itself</td>
<td>72</td>
<td>(87)</td>
</tr>
<tr>
<td>9</td>
<td>TP53</td>
<td>Mutation</td>
<td>VSCC VIN,LS</td>
<td>ND</td>
<td>- / -</td>
<td>6/0; Mutation found also in dysplastic mucosa.</td>
<td>NI Possibly early event in transformation</td>
<td>10</td>
<td>(81)</td>
</tr>
<tr>
<td>10</td>
<td>EGFR amplification</td>
<td>VSCC</td>
<td>LIV</td>
<td>- / -</td>
<td>6/5; *correlation with decreased survival</td>
<td>*HPV not present with gene amplification</td>
<td>51</td>
<td>(80)</td>
<td></td>
</tr>
</tbody>
</table>

Histological type: VSCC–vulvar squamous cell carcinoma, VIN– vulvar intraepithelial neoplasia, LS–lichen sclerosus, NI–not investigated, NF–not found, *–statistically significant, LOH–loss of heterozygosity

pathologic markers performed by Knopp and co-workers (45) revealed that p21WAF/CIP expression may not be used as pathologic marker. On the other hand Zawislak et al. (46) lately showed a down-regulation of p21WAF/CIP in VIN patients receiving photodynamic therapy. In the light of this further studies are necessary to clarify the prognostic utility of p21 WAF/CIP expression level in women with early- and advanced-stage vulvar carcinomas.

3.6. 14-3-3 sigma - a negative vulvar cancer regulator

The 14-3-3 sigma protein is regulated in a p53-dependent manner (47). It acts as a negative cell-cycle regulator by binding to CDK and sequestering the B1-CDC2 complex (G2/M cell cycle arrest). Interestingly, 14-3-3 sigma, a downstream target of p53 (48). Furthermore, downregulation of 14-3-3 sigma alone was a sufficient factor for human keratinocytes to bypass replicative senescence (at the same time lowering the expression of p16INK4A), thus promoting immortalization of the primary fibroblast (49). Methylation of TP53 downstream gene target, 14-3-3sigma, was reported in both premalignant lesions and more than half of VCSS tumors, irrespective of HPV and TP53 status (50). At the same time, it has been observed in half of VIN III cases, but none of VIN I and normal tissue samples, suggesting this genetic alteration is an early event during vulvar carcinogenesis. Furthermore, in VSCC and VIN III samples 14-3-3 sigma gene methylation is frequently accompanied by the inactivation of p16INK4a (50) (Table 3, 4).

In the study of Wang et al. (51) 14-3-3 sigma expression (both cytoplasmic and nucleolar) has been shown to correlate with larger tumor size and deep myometrial invasion. However, no association with clinical outcome has been observed, which indicates that the expression level has no prognostic value. The pattern of altered 14-3-3 sigma cellular localization (from exclusively cytoplasmic in normal cells to cytoplasm and nucleus in cancer cells) also appears to have no significant correlation with clinical parameters (51).

3.7. INK4a/ARF as vulvar cancer markers

The INK4a/ARF locus encodes two distinct proteins, p16INK4a and p14ARF, which are important RB- and TP53-pathway players (52). p16INK4a methylation was found in both VINs and vulvar cancer cells, suggesting that this event occurs early in the VSCC development (29). On the other hand, its overexpression, reported in a substantial subset of VSCC cases, was found to be associated with HPV infection, enabling to distinguish HPV related and unrelated neoplasms (53). Its high level of expression may be indirectly caused by the E7 protein, as it inactivates pRb (12, 54). This results in both the abrogation of negative feedback loop for p16INK4a and the enhancement of its transcription by released E2F. High nuclear
## Table 4. Protein expression investigated in VSCC and cancer associated lesions

<table>
<thead>
<tr>
<th>Protein</th>
<th>Expression</th>
<th>HPV</th>
<th>Description</th>
<th>Survival/prognosis</th>
<th>Remarks</th>
<th>No</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 TP53</td>
<td>Over</td>
<td>ND</td>
<td>VSCC,LS, SH</td>
<td>70% of VSCC, 40% of LS, 10% of SH</td>
<td>NF</td>
<td>Overexpression in mutated and wild type</td>
<td>27</td>
</tr>
<tr>
<td>2 TP53</td>
<td>Over</td>
<td>I-V</td>
<td>VSCC,K,W,B</td>
<td>53% of VSCCs No correlation with subtype</td>
<td>NF (for p53 and HPV)</td>
<td>Irrespective of HPV status; only when p53 highly positive; HPV+ *more frequent</td>
<td>66</td>
</tr>
<tr>
<td>3 TP53</td>
<td>Over</td>
<td>I-V</td>
<td>VSCC, VIN, LS</td>
<td>60% of VSCCs 32% of adjacent lesions 0% of normal skin *correlates with tumor grade</td>
<td>No correlation with actuarial survival or disease free survival, but trend toward shorter disease free interval in those with lymph node metastases</td>
<td>115</td>
<td>(31)</td>
</tr>
<tr>
<td>4 TP53</td>
<td>Over</td>
<td>I-V</td>
<td>VSCC, K, W, B</td>
<td>120/217</td>
<td>NF</td>
<td>*inverse correlation between TP53 expression and presence of HPV; 23% of HPV+, 64% of HPV-</td>
<td>217</td>
</tr>
<tr>
<td>5 TP53</td>
<td>Over</td>
<td>ND</td>
<td>VSCC, K, W, B</td>
<td>12/21</td>
<td>NI</td>
<td>20% of HPV+, 68.8% in HPV-</td>
<td>21</td>
</tr>
<tr>
<td>6 14-3-3</td>
<td>High</td>
<td>I-V</td>
<td>VSCC</td>
<td>36% of VSCCs</td>
<td>*correlation with large tumor diameter and deep invasion</td>
<td>No correlation</td>
<td>302</td>
</tr>
<tr>
<td>7 P14**</td>
<td>High</td>
<td>I-V</td>
<td>VSCC, K, W, B</td>
<td>36%+</td>
<td>*more frequent at lower age *lower risk of lymph node metastases</td>
<td>Related to p53 low expression 85% of HPV+, 15% of HPV- *correlates with HPV+</td>
<td>217</td>
</tr>
<tr>
<td>8 P16</td>
<td>High</td>
<td>I-V</td>
<td>VSCC,K,W,B</td>
<td>36%+</td>
<td>*correlation as with better survival</td>
<td>Related to p53 low expression 85% of HPV+, 15% of HPV- *correlates with HPV+</td>
<td>217</td>
</tr>
<tr>
<td>9 P16</td>
<td>High</td>
<td>I-V</td>
<td>VSCC,B,W,K,VIN</td>
<td>2 different patterns of staining for B,W (VINIII as well) and K subtypes</td>
<td>NF</td>
<td>*correlates with HPV status – 2 patterns of expression allow to distinguish HPV+ and HPV negative cancers shows relation of HPV to cancer subtype</td>
<td>177</td>
</tr>
<tr>
<td>10 P16</td>
<td>High</td>
<td>ND</td>
<td>VSCC,K,W,B</td>
<td>11/21</td>
<td>NI</td>
<td>7 HPV-</td>
<td>21</td>
</tr>
<tr>
<td>11 P21</td>
<td>High</td>
<td>I-V</td>
<td>VSCC</td>
<td>- -</td>
<td>Increase in lymph node metastases in comparison to primary tumor</td>
<td>NF</td>
<td>Possible role in metastasis</td>
</tr>
<tr>
<td>12 P21</td>
<td>High</td>
<td>I-V</td>
<td>VSCC,K,W,B</td>
<td>- -</td>
<td>*correlates with shorter disease free survival for FIGO I&amp;II;</td>
<td>*correlates with HPV+</td>
<td>217</td>
</tr>
<tr>
<td>13 P27</td>
<td>Low</td>
<td>I-V</td>
<td>VSCC</td>
<td>- -</td>
<td>Present in 70% of tumors</td>
<td>NF</td>
<td>*Correlation with p53 expression *correlates with HPV+</td>
</tr>
<tr>
<td>14 P27</td>
<td>Loss</td>
<td>I-V</td>
<td>VSCC,K,NK,H,W,VIN,LS,SH</td>
<td>31% of VSCCs and 14% of VIN,</td>
<td>NF</td>
<td>Trend toward correlation with poor differentiation</td>
<td>51</td>
</tr>
<tr>
<td>15 pRB</td>
<td>Loss</td>
<td>I-V</td>
<td>VSCC,VIN,LS</td>
<td>21% of VSCCs, 0% of VIN, 0% of LS</td>
<td>NF</td>
<td>Loss of pRB could be secondary event in the transformation</td>
<td>72</td>
</tr>
<tr>
<td>16 pRB</td>
<td>Loss</td>
<td>I-V</td>
<td>VSCC,VIN,LS,SH</td>
<td>37% of VSCCs, 46% of VIN, 42% of LS, 62% of SH *correlates with poorly differentiated tumors</td>
<td>NF</td>
<td>Changes of expression may be an early event in VSCC formation</td>
<td>57</td>
</tr>
<tr>
<td>17 pRB</td>
<td>Loss</td>
<td>ND</td>
<td>VSCC</td>
<td>1/21</td>
<td>NI</td>
<td>Possible role in vulvar carcinogenesis</td>
<td>21</td>
</tr>
<tr>
<td>18 pRB/ p130</td>
<td>Loss</td>
<td>I-V</td>
<td>VSCC,K,NK,H,W,VIN,LS</td>
<td>57% of VSCCs 14% of VIN, *decrease from normal through VIN to VSCCs,</td>
<td>NF</td>
<td>Possible role in vulvar carcinogenesis</td>
<td>51</td>
</tr>
<tr>
<td>19 A</td>
<td>High</td>
<td>I-V</td>
<td>VSCC</td>
<td>70% of tumors</td>
<td>NF</td>
<td>*correlation with p16,</td>
<td>217</td>
</tr>
</tbody>
</table>
expression of p16\textsuperscript{INK4a} was found in 31\% of all investigated cases, 74\% were from patients below 54 years of age (43). Moreover, the p16\textsuperscript{INK4a} overexpression may indicate a lower risk of lymph node metastases and a decreased risk of vulvar cancer-related deaths (43). Expression of p16\textsuperscript{INK4a} may be used as a marker in differential diagnosis between VINs and malignant lesions originated from the vagina (55). Finally p16\textsuperscript{INK4A} is generally highly expressed in HPV-associated VIN lesions and V SCC but its expression is not up-regulated in VINs and V SCC lacking HPV (56).

In previous reports on vulvar cancer, the level of expression of p14\textsuperscript{ARF} has been shown to be closely related to p73 expression and p53 function loss (57). High expression of p14\textsuperscript{ARF} was found in over 1/3 of VSCCs, showing correlation with disease-free survival in patients without HPV infection and high expression of p53 (18), suggesting it to be a potential prognostic marker of V SCC. Finally, Soufri and co-workers (58) demonstrated that methylation of INK4A or ARF genes is not frequent event in external genital carcinomas and their precursor lesions, including VIN and V SCC cases. However, p14\textsuperscript{ARF} expression was found to be unrelated to clinical outcomes of patients affected by vulvar cancer (45).

### 3.8. Cyclins as vulvar cancer regulators
Cyclins are regulatory proteins involved in cell-cycle progression (33, 59). Their aberrant expression was commonly reported in a number of human neoplasm types and cancer cell-lines [for a review see (60-62)]. Investigation of cyclins D1, D3, E and A by Knopp and co-workers (63), showed their overexpression in 26\%, 27\%, 18\% and 70\% of vulvar cancers, respectively. The expression of all cyclins was significantly correlated with HPV status (18). Interestingly, the abnormal expression of cyclin D1 in squamous vulvar cell carcinomas was most pronounced, when compared with adjacent normal epithelium (64).

Although an association of high level of cyclin A with tumor parameters (diameter, depth of myoinvasion or histological differentiation) has been found, suggesting it may be involved in the tumorigenesis of the vulva, none of the investigated proteins turned out to be of any prognostic significance (63). Another investigations from the same group showed increased expression of D1 in lymph node metastases, suggesting its role in cancer progression (44). The exact role of cyclins in vulvar cancer still needs to be elucidated, as these proteins seem to play a key role in the progression of this type of cancer.

### 3.9. pRb alterations in vulvar carcinogenesis
pRb protein, a member of the pRb-pathway (pRb-cyclin D1-p16\textsuperscript{INK4A}-cdk4/6), has been intensively evaluated in various human neoplasms originating from female genital tract organs (65) [for a review see (12)]. Loss of expression of proteins from the pRb family is a frequent event in V SCC samples (17, 64, 66). It has also been reported in premalignant lesions (decreasing from normal...
through premalignant to cancerous tissue), suggesting it to be an early event in vulvar carcinogenesis (66). Loss of its expression may be caused by the interaction with HPV E7, as this oncoprotein promotes its degradation (26, 60). This low pRb expression phenotype was associated with poor differentiation of the tumor (64). Moreover, the combination of the lack of p16INK4 and/or Rb expression increased from benign lesions, to invasive squamous cell carcinoma. Such observation supports the idea that alterations of p16INK4 or Rb expression could be important events in progression of the disease. (67).

3.10. Matrix metalloproteinases as predictors of tumor invasiveness

MMP-12, known as macrophage metalloelastase or macrophage elastase, is the protein involved in extracellular matrix degradation. MMP-12 expression in vulvar epithelial cancer cells has been reported to significantly correlate with histological de-differentiation. At the same time, the expression of MMP-12 in tumor infiltrating macrophages has been more abundant in well-differentiated tumor and has not correlated with blood vessel status. However, MMP-12 expression has not correlated with either lymph node status or patient outcome (68). In addition, MMP-2 expression has been reported to be associated with higher lymph node positivity and worse survival. (69)

3.11. Genomic abnormalities in vulvar cancer

Several studies evaluated genomic abnormalities in vulvar cancer cell-lines and VSCC (28, 32, 70-76). For example, among genomic alterations found by Lee et al. (72), the most frequent disturbances found were: loss in both 4p and 3p, gain of 3q and 8p. In addition, amplification of 3q was reported in both VSCC and LS suggesting it to be an early event in VSCC formation (73). Notably, a high level amplification of 3q and 5p was found in vulvar tumors, indicating a pivotal role of these genetic abnormalities in vulvar carcinogenesis (76).

3.12. Aberrant genome methylation in vulvar cancer

Epigenetic silencing by CpG islands methylation was investigated in multiple vulvar cancer cell-lines. TSG (Tumor Suppressor Gene) methylation confers loss of TSG function, thus contributing to neoplasm development. Aberrant methylation pattern for nine TSGs was demonstrated in 11/13 of squamous vulvar cancer cell-lines. The most frequently distributed pattern was observed on TP73 gene (involved in cell cycle regulation, apoptosis). The other methylated genes are IGSF4, DAPK1, FHIT, VHL, APC, ESR1, CDKN2B and GSTP1 (77). There is much to be done in the field of epigenetics of vulvar carcinoma.

3.13. Immune system deficiencies in vulvar cancer

Immune system deregulation is a characteristic feature of LS, prevalently associated with the keratinizing type of VC (10). Monoclonal gamma-TCR-rearrangement has been found only in lesions from LS patients and squamous cell carcinoma of the vulva arising in LS. At the same time it was not observed among VS samples from patients without LS. Furthermore, lesions with this rearrangement have shown an altered profile of lichenoid infiltrate of CD4 T-cells, B-cells, and fascin-expressing dendritic cells. The infiltrate profile has been suggested to be a specific cellular immune response to the proliferating T clones or some LS-specific antigen (yet undefined), thus creating a local, deregulated, immune environment that facilitates the development of SCC in LS (78). On the other hand, the development of the VSCC is observed in increasing numbers of immunocompromised (due to immunosuppressive therapies or HIV infections) patients. This trend confirms that immune dysfunctions must be considered as a factor of vulvar neoplastic transformation (79).

3.14. Other potential molecular markers

Many other genes and proteins have been investigated in the field of vulvar cancer. Most interesting results were obtained for EGFR, PTEN, ZNF652, Laminin-5g2 and CHK2. EGFR gene amplification correlates with decreased patients’ survival and absence of high-risk HPV infection, thus possibly indicating HPV-independent vulvar carcinogenesis. No mutations were found and the percentage of patients with amplification was lower than the average for epithelial cancer. The study of Growdon et al. suggested that these patients may benefit from small EGFR inhibiting therapeutics (80). PTEN gene point mutations may be considered as an early event in vulvar carcinoma development. These were identified in vulvar carcinoma, in both cancer tissue and corresponding dysplastic mucosa (81). However, no study investigating the expression pattern and the prognostic value of PTEN mutation has yet been conducted. ZNF652- tumor suppressor protein has been shown to be down-regulated in cases of VIN II and VIN III as well as in VC patients (in comparison to normal tissue). It has been suggested that ZNF652 plays a role in the pathogenesis of the VSCC, however, the level of the protein has not been found to correlate with the prognosis of the disease (82). Laminin-5g2-chain has been reported to be present only in VSCC samples, but not in adjacent precancerous lesions, suggesting it may be useful in distinguishing the two types of alterations (73). CHK2 mutations are found in a small percentage of vulvar cancers, coexisting with TP53 mutations (83).

4. SUMMARY AND PERSPECTIVES

All molecular changes reported here provide some insight into vulvar cancer pathogenesis. However, the data are still incomplete. HPV infection is probably the most important etiological factor in the development of VSCC. Although some of the alterations have been shown to correspond to clinical parameters, there is not enough evidence to use any of them as useful molecular markers directly applicable in the clinical practice. This may be explained by the fact that a relatively low frequency of VSCC, in most cases making it difficult to collect a representative sample. Moreover, there are a few studies on the same topic and comparison of the results from different studies remains problematic, as frequently different
Endometrial biomarker

research models are used. The data on molecular susceptibility for VSCC development is still missing. There is a great need for a novel approach to fight vulvar cancer, and molecular medicine may provide an interesting option for that (84, 85).

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6. REFERENCES


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Key Words: Vulvar Cancer, Molecular markers, Carcinogenesis, VIN, Lichen Sclerosus, VSCC, Review

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