[Frontiers in Bioscience S3, 136-144, January 1, 2011]

Vulvar cancer as a target for molecular medicine

Aleksandra Klemba[1,2], Wojciech Kukwa[3], Andrzej Semczuk[4], Anna M. Czarnecka[1,2]

[1]Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, 5a Pawinskiego Street, 02-106, Warsaw, Poland, [2]Laboratory of Molecular Oncology, Department of Oncology, Military Institute of Medicine, ul. Szaserow 128, 04-141 Warsaw, Poland, [3]Department of Otolaryngology, Czerniakowski Hospital, Medical University of Warsaw, 19/25 Stępinska Street, 00-739 Warsaw, Poland, [4]II[ND] Department of Gynecology, Lublin Medical University, Lublin, Poland

TABLE OF CONTENTS

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.

Table 1. General features of two basic subtypes of VSCC

Feature	HPV –related	HPV-unrelated
Age of diagnosis	Younger	Elderly
Subtype	Basaloid verrucoid	(non)keratinizing
Prognosis	Better	Worse

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

Feature	Usual VIN (uVIN)	Differentiated VIN (dVIN)
Occurrence/incidence	Common	Less common
Age of patients	Younger	Elderly(70+)
HPV status	Positive (especially HPV 16)	Negative
Usual lesions	Multifocal	Unifocal
Possibility of progression to SCC	3-4%	Rapid progression- of ten
Subtype of VSSC developed	Warty, Basaloid	Keratinizing/non keratinizing
Other:		More frequent among VC patients
Molecular characteristics (9)		
HPV (DNA) $p16^{INK4}$ MIB1 p53	positive positive positive negative	negative negative negative positive
Other	uVIN+SCC- 53 y.o. uVIN-SCC- 36 y.o.	dVIN+SCC-74 y.o. dVIN-SCC-not present

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, $p16^{INK4a}$ and MIB-1 (9). The first two (p53, $p16^{INK4a}$) are proteins involved in important signaling pathways (12, 33). The key molecules of those pathway are also $p14^{ARF}$, 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-ocurring 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. $p21^{WAF/CIP}$ 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. $p21^{WAF/CIP}$ by 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 $p21^{WAF/CIP}$ 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 $p21^{WAF/CIP}$ was significantly correlated with decreased disease-free survival of patients staged I/II (43). In the study of advanced VSCC cases, increased $p21^{WAF/CIP}$ expression was reported in lymph node metastases, suggesting a metastasis moderator role of this protein (44). Recently, however, a review of molecular

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

	Gene	Aberration	Histological type/lesion	FIGO	HPV	TP53	Description	Survival/ prognosis	Remarks	No	Ref
1	*TP53*	Mutation	VSCCLS SH	ND	-	/	77.8% of VSCCs, 46.7% of LS , 22.2% SH	NI	Mutation in non neoplastic lesions may lead to VSCC development	27 15 9	(86)
2	*TP53*	LOH	VSCC	I-IV	+ 16	/	21/36, *LOH more frequent in case of 72RP that in 72RR	NI	LOH irrespective of HPV status	36	(29)
3	*TP53*	Mutation	VSCC	I-IV	+ 16	/	21/36	NI	TP53 mutation more common in HPV negative	36	(29)
4	*TP53*	Mutation	VSCC	I-IV	-	/	12/38 More common in advanced stages (III I IV)	*correlation with relapse free survival *correlation with overall survival		38	(35)
5	*TP53*	Mutation	VSCC K,NK, W,B	ND	+	/	13/21	NI	40% in HPV+, 68.8% in HPV- HPV not in K and NK	21	(17)
6	*14-3-3*	Methylation (transcriptional silencing)	VSSC VIN	ND	+ 16	+	60% of VSCC, Present in late VIN	NI	Methylation of 14-3-3 associated with methylation of p16Ink4a/No mutations Possibly early event in VC development Irrespective of p53 status and HVP	36 32	(50)
7	*p16Ink4a*	Methylation (& transcriptional silencing)	VSSCVIN	ND	+ 16	+	13/36 of VSCC; Present in late VIN	NI	Methylation of p16Ink4a coexists with methylation of 14-3-3 /Possibly early event in VC development/Irrespective of p53 status and HVP	36 32	(50)
8	*P16Ink4a*	Methylation	VSCC VIN,LS	I-IV	-	-	68% of VC, 69,2% of VIN, 42.8% of LS	NI	Possibly an early event in transformation, but insufficient by itself	72	(87)
9	*PTEN*	Mutation	VSCC	ND	-	-	6/10;Mutation found also in dysplastic mucosa,	NI	Possibly early event in VC formation	10	(81)
10	*EGFR*	amplification	VSCC	I-IV	+	-	6/51, No correlation with stage/ lymph node status	*correlation with decreased survival	*HPV not present with gene amplification	51	(80)

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 $p21^{WAF/CIP}$ expression may not be used as pathologic marker. On the other hand Zawislak *et al.* (46) lately showed a down-regulation of $p21^{WAF/CIP}$ 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, has also been shown to positively regulate p53 activity, by stabilizing its expression and enhancing its transcriptional activity, thus contributing to tumor suppression (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 $p16^{INK4A}$), 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 $p16^{INK4a}$ (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 of 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, $p16^{INK4a}$ and $p14^{ARF}$, which are important RB- and TP53-pathway players (52). $p16^{INK4a}$ 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 $p16^{INK4a}$ and the enhancement of its transcription by released E2F. High nuclear

Table 4. Protein expression investigated in VSCC and cancer associated lesions

	Protein	Expression	FIGO	Histological type	HPV	TP53	Description	Survival/prognosis	Remarks	No	Ref
1	TP53	Over expression	ND	VSCCLS SH	-	/	70% of VSCC, 40% of LS, 10% of SH	NF	Overexpression in mutated and wild type	27 15 9	(86)
2	TP53	Over expression	I-IV	VSCC K,W,B	+	/	53% of VSCCs No correlation with subtype	NF (for p53 and HPV)	Irrespective of HPV status; only when p53 highly positive- HPV- *more frequent	66	(30)
3	TP53	Over expression	I-IV	VSCC VIN LS	-	/	68% of VSCCs 32% of adjacent lesions 0% of normal skin *correlates with tumor grade	No correlation with actuarial survival or disease free survival, but trend toward shorter disease- free interval in those with lymph node metastates		115	(31)
4	TP53	Over expression	I-IV	VSCC, NK, K, W,B	+	/	120/217	NF	*inverse correlation between TP53 expression and presence of HPV 23%of HPV+, 64% of HPV-	217	(18)
5	TP53	Over expression	ND	VSCC NK, K, W,B	+	/	12/21	NI	20% of HPV+, 68.8% in HPV-	21	(17)
6	14-3-3	High expression	I-IV	VSCC	+	+	*correlation with large tumor diameter and deep invasion	NF	No correlation p14, p16, p21, p27, p53 and cyclins A1, D1, D3, E, HPV. In a small subset of cancer (25%) may be involved in carcinogenesis	302	(51)
7	$P14^{Arf}$	High expression	I-IV	VSCC,NK, K, W,B	+	+	36% of VSCCs	*correlation of p14 presence with disease free survival In HPV- and high p53 cases	64% of HPV+, 28% of HPV- *correlates with HPV presence	217	(18)
8	P16	High expression	I-IV	VSCC NK, K, W,B	+	+	36%, *more frequent at lower age *lower risk of lymph node metastasis	*correlation/as with better survival	Related to p53 low expression 85% of HPV+, 15% of HPV- *correlates with HVP+	217	(43) (18)
9	P16	High expression	I-IV	VSCC B,W,K,VIN	+	-	2 different patterns of staining for B,W (VINIII as well) and K subtypes	NI	*correlates with HPV status – 2 patterns of expression allow to distinguish HPV+ and HPV negative cancers shows relation of HPV to cancer subtype	177	(53)
10	P16	High expression	ND	VSCC,K NK,W,B	+	+	11/21	NI	7 HPV-	21	(17)
11	P21	High expression	I-IV	VSCC	-	-	Increase in lymph node metastates in comparison to primary tumor	NI	Possible role in metastasis	53	(44)
12	P21	High expression	I-IV	VSCC,K NK, W,B	+	+	Low expression in 70% of well differentiated tumors	*correlates with shorter disease free survival for FIGO I&II;	*correlates with HPV + *correlates with p53	217	(43) (18)
13	P27	Low expression	I-IV	VSCC	+	+	Present in 70% of tumors	NF	*Correlation with p53 expression *correlates with HPV +	217	(43) (18)
14	P27	Loss of expression	I-IV	VSCC,K, NK,B,W,V VIN,LS,SH	-	-	31% of VSCCs and 14% of VIN,	NI	Trend toward correlation with poor differentiation	51	(66)
15	pRB	Loss of expression	I-IV	VSCC VIN LS	-	-	21% of VSCCs, 0% of VIN, 0% of LS		Loss of pRB could be secondary event in the transformation	72	(87)
16	pRB	Loss of expression	I-IV	VSCC VIN LS SH	-	-	37% of VSCC , 46% of VIN 42% of LS, 62% of SH 0% in normal epithelium *correlation with poorly differentiated tumors	NF	Changes of expression may be an early event I VSCC formation	57 11 19 3	(64)
17	pRB	Loss of expression	ND	VSCC	+	+	3/21	NI		21	(17)
18	pRB2/ p130	Loss of expression	I-IV	VSCC K,NK,B.W,V,VIN LS	-	-	57% of VSCCs 14% of VIN, *decrease from normal through VIN to VSCCs,	NI	Possible role in vulvar carcinogenesis	51	(66)
19	A	High	I-	VSCC	+	+	70% of tumors	NF	*correlation with p16,	217	(63)

		expression	IV				*Correlation with tumor diameter, tumor differentiation, depth of invasion		p21; *correlation with HPV+ Possibly early event in VC formation		(18)
20	D1	Over expression	I-IV	VSCC VIN LS H	-	-	51% of VSCC , 18% of VIN 50% of LS, 31% of SH 0% in normal epithelium, *Correlates with depth of invasion,	NF	Changes of expression may be an early event in VSCC formation	57 11 19 3	(64)
21	D1	Over expression	I-IV	VSCC VIN LS	-	-	21% of VSCC, 30,8% of VIN, 0% of LS	NI	Associated with p16 methylation, both may contribute to transformation	72	(87)
22	D1	Low expression	I-IV	VSCC	+	+	167/217	NF	*correlation with p16,p21,p53 *correlation of low expression and HPV+	217	(63) (18)
23	D1	High expression	I-IV	VSCC	-	-	Increase in lymph node metastases in comparison to primary tumor	NI		53	(44)
24	D3	Low espression	I-IV	VSCC	+	+	157/217	NF	*correlation with p16,p53 *correlation with HPV+	224	(63)
25	E	Low expression	I-IV	VSCC	+	+	178/217	NF	96% of HPV+ *correlation with HVP+	217	(18)
26	EGFR	High expression	I-IV	VSCC	+	-	*correlation with advanced stage	*correlation with worse survival		51	(80)
27	ZNF652	Reduced expression	I-IV	VSCC VIN	+	+	Reduced in 25% of VIN II, 100% VIN III, 50% of VSCC, reduction;	NF	no correlation with p16, p21, p27, p53 cyclins A, D1, D3, E, HPV possibly early event in VC development	217 22	(82)
28	Laminin-5g2-chain	expression	ND	VSCC VIN	-	-	Present in VSCC, not in adjacent lesions	NI	Enables to distinguish two types of tissue		(73)

Histological type: VSCC-vulvar squamous cell carcinoma, VIN- vulvar intraepithelial neoplasia, LS-lichen sclerosus, NI-not investigated, NF-not found, *-statistically significant

expression of $p16^{INK4a}$ was found in 31% of all investigated cases, 74% were from patients below 54 years of age (43). Moreover, the $p16^{INK4a}$ overexpression may indicate a lower risk of lymph node metastases and a decreased risk of vulvar cancer-related deaths (43). Expression of $p16^{INK4A}$ may be used as a marker in differential diagnosis between VINs and malignant lesions originated from the vagina (55). Finally $p16^{INK4A}$ is generally highly expressed in HPV-associated VIN lesions and VSCC but its expression is not up-regulated in VINs and VSCC lacking HPV (56).

In previous reports on vulvar cancer, the level of expression of $p14^{ARF}$ has been shown to be closely related to p73 expression and p53 function loss (57). High expression of $p14^{ARF}$ 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 VSCC. Finally, Soufir 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 VSCC cases. However, $p14^{ARF}$ 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-$p16^{INK4A}$-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 VSCC 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 staining enabled to distinguish VSCC from vulvar lesions (VIN, LS) (69). No further studies have been conducted in this field, so that no general conclusion can be withdrawn about the role of MMPs in vulvar carcinoma.

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 Jee *et al.* (72), the most frequent disturbances found were: loss in both 4p and 3p and 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 Supressor 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 inifiltrate 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 clone 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

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).

5. AKNOWLEDGEMENTS

The authors thank dr Przemyslaw Tomalski, PhD, from the University of East London, London, UK for critical reading of the manuscript and editing the final version of the text.

6. REFERENCES

1. M. J. Quinn, A. d'Onofrio, B. Moller, R. Black, C. Martinez-Garcia, H. Moller, M. Rahu, C. Robertson, L. J. Schouten, C. La Vecchia and P. Boyle: Cancer mortality trends in the EU and acceding countries up to 2015. *Ann Oncol*, 14(7), 1148-52 (2003)

2. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu and M. J. Thun: Cancer statistics, 2009. CA Cancer J Clin, 59(4), 225-49 (2009)

3. E. A. Joura, A. Losch, M. G. Haider-Angeler, G. Breitenecker and S. Leodolter: Trends in vulvar neoplasia. Increasing incidence of vulvar intraepithelial neoplasia and squamous cell carcinoma of the vulva in young women. *J Reprod Med*, 45(8), 613-5 (2000)

4. U. Hording, J. Junge, S. Daugaard, F. Lundvall, H. Poulsen and J. E. Bock: Vulvar squamous cell carcinoma and papillomaviruses: indications for two different etiologies. *Gynecol Oncol*, 52(2), 241-6 (1994)

5. W. G. McCluggage: Recent developments in vulvovaginal pathology. *Histopathology*, 54(2), 156-73 (2009) doi:HIS3098

6. E. K. Wilkinson, B; Lynch,PJ: Report of the ISSVD. Terminology Committee. *J Reprod Med*(31) (1986)

7. G. Bręborowicz: Położnictwo i ginekologia. Warszawa (2008)

8. M. Sideri, R. W. Jones, E. J. Wilkinson, M. Preti, D. S. Heller, J. Scurry, H. Haefner and S. Neill: Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. *J Reprod Med*, 50(11), 807-10 (2005)

9. B. M. Hoevenaars, I. A. van der Avoort, P. C. de Wilde, L. F. Massuger, W. J. Melchers, J. A. de Hullu and J. Bulten: A panel of p16(INK4A), MIB1 and p53 proteins can distinguish between the 2 pathways leading to vulvar squamous cell carcinoma. *Int J Cancer*, 123(12), 2767-73 (2008)

10. A. Olsson, P. Selva-Nayagam and M. K. Oehler: Postmenopausal vulval disease. *Menopause Int*, 14(4), 169-72 (2008)

11. J. S. Smith, D. M. Backes, B. E. Hoots, R. J. Kurman and J. M. Pimenta: Human papillomavirus type-distribution in vulvar and vaginal cancers and their associated precursors. *Obstet Gynecol*, 113(4), 917-24 (2009)

12. K. Milde-Langosch and S. Riethdorf: Role of cell-cycle regulatory proteins in gynecological cancer. *J Cell Physiol*, 196(2), 224-44 (2003)

13. J. P. Kunjoonju, M. Raitanen, S. Grenman, N. Tiwari and M. J. Worsham: Identification of individual genes altered in squamous cell carcinoma of the vulva. *Genes Chromosomes Cancer*, 44(2), 185-93 (2005)

14. I. A. van der Avoort, H. Shirango, B. M. Hoevenaars, J. M. Grefte, J. A. de Hullu, P. C. de Wilde, J. Bulten, W. J. Melchers and L. F. Massuger: Vulvar squamous cell carcinoma is a multifactorial disease following two separate and independent pathways. *Int J Gynecol Pathol*, 25(1), 22-9 (2006)

15. D. G. Allen, A. M. Hutchins, F. Hammet, D. J. White, J. P. Scurry, S. N. Tabrizi, S. M. Garland and J. E. Armes: Genetic aberrations detected by comparative genomic hybridisation in vulvar cancers. *Br J Cancer*, 86(6), 924-8 (2002)

16. D. Subramanya and P. D. Grivas: HPV and cervical cancer: updates on an established relationship. *Postgrad Med*, 120(4), 7-13 (2008) doi:10.3810/pgm.2008.11.1928

17. M. Osakabe, M. Hayashi, Y. Katayama, I. Emura, K. Nemoto, H. Umezu, K. Saitoh and T. Motoyama: Characteristics of vulvar squamous cell carcinoma in Japanese women. *Pathol Int*, 57(6), 322-7 (2007)

18. S. Knopp, J. M. Nesland, C. Trope and R. Holm: p14ARF, a prognostic predictor in HPV-negative vulvar carcinoma. *Am J Clin Pathol*, 126(2), 266-76 (2006)

19. H. zur Hausen and E. M. de Villiers: Human papillomaviruses. *Annu Rev Microbiol*, 48, 427-47 (1994)

20. H. R. McMurray, D. Nguyen, T. F. Westbrook and D. J. McAnce: Biology of human papillomaviruses. *Int J Exp Pathol*, 82(1), 15-33 (2001)

21. M. E. McLaughlin-Drubin and K. Munger: Oncogenic activities of human papillomaviruses. *Virus Res* (2009) doi:S0168-1702(09)00221-4

22. M. S. Barbosa, W. C. Vass, D. R. Lowy and J. T. Schiller: *In vitro* biological activities of the E6 and E7 genes vary among human papillomaviruses of different oncogenic potential. *J Virol*, 65(1), 292-8 (1991)

23. A. Bernat, P. Massimi and L. Banks: Complementation of a p300/CBP defective-binding mutant of adenovirus E1a by human papillomavirus E6 proteins. *J Gen Virol*, 83(Pt 4), 829-33 (2002)

24. X. Liu, A. Dakic, Y. Zhang, Y. Dai, R. Chen and R. Schlegel: HPV E6 protein interacts physically and functionally with the cellular telomerase complex. *Proc Natl Acad Sci U S A*, 106(44), 18780-5 (2009)

25. R. Hesketh: The oncogene and tumous suppressor gene. Academic Press, San Diego London, Boston New York Sydney Tokyo Toronto (1997)

26. E. A. Sisk and E. S. Robertson: Clinical implications of human papillomavirus infection. *Front Biosci*, 7, e77-84 (2002)

27. N. Wentzensen, S. Vinokurova and M. von Knebel Doeberitz: Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. *Cancer Res*, 64(11), 3878-84 (2004)

28. A. P. Pinto, M. C. Lin, G. L. Mutter, D. Sun, L. L. Villa and C. P. Crum: Allelic loss in human papillomavirus-positive and -negative vulvar squamous cell carcinomas. *Am J Pathol*, 154(4), 1009-15 (1999)

29. L. A. Brooks, J. A. Tidy, B. Gusterson, L. Hiller, J. O'Nions, M. Gasco, M. C. Marin, P. J. Farrell, W. G. Kaelin, Jr. and T. Crook: Preferential retention of codon 72 arginine p53 in squamous cell carcinomas of the vulva occurs in cancers positive and negative for human papillomavirus. *Cancer Res*, 60(24), 6875-7 (2000)

30. M. J. Kagie, G. G. Kenter, R. A. Tollenaar, J. Hermans, J. B. Trimbos and G. J. Fleuren: p53 protein overexpression is common and independent of human papillomavirus infection in squamous cell carcinoma of the vulva. *Cancer*, 80(7), 1228-33 (1997)

31. D. T. McConnell, I. D. Miller, D. E. Parkin and G. I. Murray: p53 protein expression in a population-based series of primary vulval squamous cell carcinoma and immediate adjacent field change. *Gynecol Oncol*, 67(3), 248-54 (1997)

32. L. C. Flowers, Wistuba, II, J. Scurry, C. Y. Muller, R. Ashfaq, D. S. Miller, J. D. Minna and A. F. Gazdar: Genetic changes during the multistage pathogenesis of human papillomavirus positive and negative vulvar carcinomas. *J Soc Gynecol Investig*, 6(4), 213-21 (1999)

33. A. Semczuk and J. A. Jakowicki: Alterations of pRb1-cyclin D1-cdk4/6-p16(INK4A) pathway in endometrial carcinogenesis. *Cancer Lett*, 203(1), 1-12 (2004)

34. C. J. Sherr and F. McCormick: The RB and p53 pathways in cancer. *Cancer Cell*, 2(2), 103-12 (2002)

35. G. Sliutz, W. Schmidt, C. Tempfer, P. Speiser, G. Gitsch, S. Eder, C. Schneeberger, C. Kainz and R. Zeillinger: Detection of p53 point mutations in primary human vulvar cancer by PCR and temperature gradient gel electrophoresis. *Gynecol Oncol*, 64(1), 93-8 (1997)

36. A. P. Pinto, A. Miron, Y. Yassin, N. Monte, T. Y. Woo, K. K. Mehra, F. Medeiros and C. P. Crum: Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. *Mod Pathol*, 23(3), 404-12 (2010)

37. A. G. Emanuels, J. Koudstaal, M. P. Burger and H. Hollema: In squamous cell carcinoma of the vulva, overexpression of p53 is a late event and neither p53 nor mdm2 expression is a useful marker to predict lymph node metastases. *Br J Cancer*, 80(1-2), 38-43 (1999)

38. G. V. Sherbet and D. Patil: Genetic abnormalities of cell proliferation, invasion and metastasis, with special reference to gynaecological cancers. *Anticancer Res*, 23(2B), 1357-71 (2003)

39. A. Semczuk, R. Schneider-Stock and W. Szewczuk: Prevalence of Allelic Loss at TP53 in Endometrial Carcinomas. *Oncology*, 78(220-228) (2010)

40. M. Tommasino, R. Accardi, S. Caldeira, W. Dong, I. Malanchi, A. Smet and I. Zehbe: The role of TP53 in Cervical carcinogenesis. *Hum Mutat*, 21(3), 307-12 (2003)

41. J. O. Funk, S. Waga, J. B. Harry, E. Espling, B. Stillman and D. A. Galloway: Inhibition of CDK activity and PCNA-dependent DNA replication by p21 is blocked by interaction with the HPV-16 E7 oncoprotein. *Genes Dev*, 11(16), 2090-100 (1997)

42. Dworakowska: The role of p53, pRb, p21WAF/CIP,PCNA, mdm2, and cyclin D1 in regulation of cell cycle and apoptosis *Onkol. Pol.*, 8(4), 223-228 (2005)

43. S. Knopp, T. Bjorge, J. M. Nesland, C. Trope, M. Scheistroen and R. Holm: p16INK4a and p21Waf1/Cip1 expression correlates with clinical outcome in vulvar carcinomas. *Gynecol Oncol*, 95(1), 37-45 (2004)

44. S. Knopp, R. Holm, T. Bjorge, C. Trope and J. M. Nesland: Cell cycle regulation in primary vulvar carcinomas and related lymph node metastases. *Histopathology*, 49(3), 311-2 (2006)

45. S. Knopp, C. Trope, J. M. Nesland and R. Holm: A review of molecular pathological markers in vulvar carcinoma: lack of application in clinical practice. *J Clin Pathol*, 62(3), 212-8 (2009)

46. A. Zawislak, R. F. Donnelly, W. G. McCluggage, J. H. Price, H. R. McClelland, A. D. Woolfson, S. Dobbs, P. Maxwell and P. A. McCarron: Clinical and immunohistochemical assessment of vulval intraepithelial neoplasia following photodynamic therapy using a novel bioadhesive patch-type system loaded with 5-aminolevulinic acid. *Photodiagnosis Photodyn Ther*, 6(1), 28-40 (2009)

47. Z. Li, J. Y. Liu and J. T. Zhang: 14-3-3sigma, the double-edged sword of human cancers. *Am J Transl Res*, 1(4), 326-40 (2009)

48. H. Y. Yang, Y. Y. Wen, C. H. Chen, G. Lozano and M. H. Lee: 14-3-3 sigma positively regulates p53 and suppresses tumor growth. *Mol Cell Biol*, 23(20), 7096-107 (2003)

49. E. Dellambra, O. Golisano, S. Bondanza, E. Siviero, P. Lacal, M. Molinari, S. D'Atri and M. De Luca: Downregulation of 14-3-3sigma prevents clonal evolution and leads to immortalization of primary human keratinocytes. *J Cell Biol*, 149(5), 1117-30 (2000)

50. M. Gasco, A. Sullivan, C. Repellin, L. Brooks, P. J. Farrell, J. A. Tidy, B. Dunne, B. Gusterson, D. J. Evans and T. Crook: Coincident inactivation of 14-3-3sigma and p16INK4a is an early event in vulval squamous neoplasia. *Oncogene*, 21(12), 1876-81 (2002)

51. Z. Wang, C. G. Trope, Z. Suo, G. Troen, G. Yang, J. M. Nesland and R. Holm: The clinicopathological and prognostic impact of 14-3-3 sigma expression on vulvar squamous cell carcinomas. *BMC Cancer*, 8, 308 (2008)

52. C. J. Sherr: The INK4a/ARF network in tumour suppression. *Nat Rev Mol Cell Biol*, 2(10), 731-7 (2001)

53. S. Riethdorf, E. F. Neffen, A. Cviko, T. Loning, C. P. Crum and L. Riethdorf: p16INK4A expression as biomarker for HPV 16-related vulvar neoplasias. *Hum Pathol* 35(12), 1477-83 (2004)

54. M. B. Moller: Molecular control of the cell cycle in cancer: biological and clinical aspects. *Dan Med Bull*, 50(2), 118-38 (2003)

55. I. Rufforny, E. J. Wilkinson, C. Liu, H. Zhu, M. Buteral and N. A. Massoll: Human papillomavirus infection and p16(INK4a) protein expression in vulvar intraepithelial neoplasia and invasive squamous cell carcinoma. *J Low Genit Tract Dis*, 9(2), 108-13 (2005)

56. C. J. O'Neill and W. G. McCluggage: p16 expression in the female genital tract and its value in diagnosis. *Adv Anat Pathol*, 13(1), 8-15 (2006)

57. J. O'Nions, L. A. Brooks, A. Sullivan, A. Bell, B. Dunne, M. Rozycka, A. Reddy, J. A. Tidy, D. Evans, P. J. Farrell, A. Evans, M. Gasco, B. Gusterson and T. Crook: p73 is over-expressed in vulval cancer principally as the Delta 2 isoform. *Br J Cancer*, 85(10), 1551-6 (2001)

58. N. Soufir, S. Queille, M. Liboutet, O. Thibaudeau, F. Bachelier, G. Delestaing, B. C. Balloy, J. Breuer, A. Janin, L. Dubertret, C. Vilmer and N. Basset-Seguin: Inactivation of the CDKN2A and the p53 tumour suppressor genes in external genital carcinomas and their precursors. *Br J Dermatol*, 156(3), 448-53 (2007)

59. M. Malumbres and M. Barbacid: Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer*, 9(3), 153-66 (2009)

60. F. Sanseverino, M. Torricelli, F. Petraglia and A. Giordano: Role of the retinoblastoma family in gynecological cancer. *Cancer Biol Ther*, 2(6), 636-41 (2003)

61. K. Vermeulen, D. R. Van Bockstaele and Z. N. Berneman: The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell Prolif*, 36(3), 131-49 (2003)

62. M. Malumbres: Cyclins and related kinases in cancer cells. *J Buon*, 12 Suppl 1, S45-52 (2007)

63. S. Knopp, T. Bjorge, J. M. Nesland, C. Trope and R. Holm: Cyclins D1, D3, E, and A in vulvar carcinoma patients. *Gynecol Oncol*, 97(3), 733-9 (2005)

64. K. J. Rolfe, J. C. Crow, E. Benjamin, W. M. Reid, A. B. Maclean and C. W. Perrett: Cyclin D1 and retinoblastoma protein in vulvar cancer and adjacent lesions. *Int J Gynecol Cancer*, 11(5), 381-6 (2001)

65. A. Semczuk, R. Schneider-Stock, R. Miturski, D. Skomra, J. Tomaszewski, A. Roessner and J. A. Jakowicki: RB protein expression in human endometrial carcinomas--an immunohistochemical study. *Pathol Res Pract*, 196(1), 41-6 (2000)

66. A. Zamparelli, V. Masciullo, A. Bovicelli, D. Santini, G. Ferrandina, C. Minimo, P. Terzano, S. Costa, C. Cinti, C. Ceccarelli, S. Mancuso, G. Scambia, L. Bovicelli and A. Giordano: Expression of cell-cycle-associated proteins pRB2/p130 and p27kip in vulvar squamous cell carcinomas. *Hum Pathol*, 32(1), 4-9 (2001)

67. M. K. Chan, T. H. Cheung, T. K. Chung, S. Y. Bao, C. L. Zhao, T. Nobori and Y. F. Wong: Expression of p16INK4 and retinoblastoma protein Rb in vulvar lesions of Chinese women. *Gynecol Oncol*, 68(2), 156-61 (1998)

68. E. Kerkela, R. Ala-aho, P. Klemi, S. Grenman, S. D. Shapiro, V. M. Kahari and U. Saarialho-Kere: Metalloelastase (MMP-12) expression by tumour cells in squamous cell carcinoma of the vulva correlates with invasiveness, while that by macrophages predicts better outcome. *J Pathol*, 198(2), 258-69 (2002)

69. A. C. Bovo, I. D. da Silva, L. C. Takita, J. Fochi, J. N. Stavale, G. Marks and G. R. de Lima: A comparative study of MMP-2 in vulvar neoplasms. *Gynecol Oncol*, 93(2), 454-7 (2004)

70. M. J. Worsham, D. L. Van Dyke, S. E. Grenman, R. Grenman, M. P. Hopkins, J. A. Roberts, K. M. Gasser, D. R. Schwartz and T. E. Carey: Consistent chromosome abnormalities in squamous cell carcinoma of the vulva. *Genes Chromosomes Cancer*, 3(6), 420-32 (1991)

71. M. C. Lin, G. L. Mutter, P. Trivijisilp, K. A. Boynton, D. Sun and C. P. Crum: Patterns of allelic loss (LOH) in vulvar squamous carcinomas and adjacent noninvasive epithelia. *Am J Pathol*, 152(5), 1313-8 (1998)

72. K. J. Jee, Y. T. Kim, K. R. Kim, H. S. Kim, A. Yan and S. Knuutila: Loss in 3p and 4p and gain of 3q are concomitant aberrations in squamous cell carcinoma of the vulva. *Mod Pathol*, 14(5), 377-81 (2001)

73. P. Stoltzfus, K. Heselmeyer-Haddad, J. Castro, N. White, C. Silfversward, K. Sjovall, N. Einhorn, K. Tryggvason, G. Auer, T. Ried and B. Nordstrom: Gain of chromosome 3q is an early and consistent genetic aberration in carcinomas of the vulva. *Int J Gynecol Cancer*, 15(1), 120-6 (2005)

74. A. N. Rosenthal, A. Ryan, D. Hopster, T. Surentheran and I. J. Jacobs: High frequency of loss of heterozygosity in vulval intraepithelial neoplasia (VIN) is associated with invasive vulval squamous cell carcinoma (VSCC). *Int J Cancer*, 94(6), 896-900 (2001)

75. M. Raitanen, M. J. Worsham, T. Lakkala, T. E. Carey, D. L. Van Dyke, R. Grenman, P. Klemi, V. Rantanen, J. Isola and S. Grenman: Characterization of 10 vulvar carcinoma cell lines by karyotyping, comparative genomic hybridization and flow cytometry. *Gynecol Oncol*, 93(1), 155-63 (2004)

76. F. Y. Huang, Y. K. Kwok, E. T. Lau, M. H. Tang, T. Y. Ng and H. Y. Ngan: Genetic abnormalities and HPV status in cervical and vulvar squamous cell carcinomas. *Cancer Genet Cytogenet*, 157(1), 42-8 (2005)

77. J. K. Stephen, K. M. Chen, M. Raitanen, S. Grenman and M. J. Worsham: DNA hypermethylation profiles in squamous cell carcinoma of the vulva. *Int J Gynecol Pathol*, 28(1), 63-75 (2009)

78. S. Regauer, O. Reich and C. Beham-Schmid: Monoclonal gamma-T-cell receptor rearrangement in vulvar lichen sclerosus and squamous cell carcinomas. *Am J Pathol*, 160(3), 1035-45 (2002)

79. S. K. Tyring: Vulvar squamous cell carcinoma: guidelines for early diagnosis and treatment. *Am J Obstet Gynecol*, 189(3 Suppl), S17-23 (2003)

80. W. B. Growdon, S. L. Boisvert, S. Akhavanfard, E. Oliva, D. C. Dias-Santagata, S. Kojiro, N. S. Horowitz, A. J. Iafrate, D. R. Borger and B. R. Rueda: Decreased survival in EGFR gene amplified vulvar carcinoma. *Gynecol Oncol*, 111(2), 289-97 (2008)

81. A. H. Holway, K. M. Rieger-Christ, W. R. Miner, J. W. Cain, J. M. Dugan, J. A. Pezza, M. L. Silverman, A. Shapter, R. McLellan and I. C. Summerhayes: Somatic mutation of PTEN in vulvar cancer. *Clin Cancer Res*, 6(8), 3228-35 (2000)

82. R. Holm, S. Knopp, R. Kumar, J. Lee, J. M. Nesland, C. Trope and D. F. Callen: Expression of ZNF652, a novel zinc finger protein, in vulvar carcinomas and its relation to prognosis. *J Clin Pathol*, 61(1), 59-63 (2008)

83. A. Reddy, M. Yuille, A. Sullivan, C. Repellin, A. Bell, J. A. Tidy, D. J. Evans, P. J. Farrell, B. Gusterson, M. Gasco and T. Crook: Analysis of CHK2 in vulval neoplasia. *Br J Cancer*, 86(5), 756-60 (2002)

84. A. M. Czarnecka, C. Campanella, G. Zummo and F. Cappello: Mitochondrial chaperones in cancer: from molecular biology to clinical diagnostics. *Cancer Biol Ther*, 5(7), 714-20 (2006)

85. A. M. Czarnecka, W. Kukwa, T. Krawczyk, A. Scinska, A. Kukwa and F. Cappello: Mitochondrial DNA mutations in cancer--from bench to bedside. *Front Biosci*, 15, 437-60 (2010)

86. K. J. Rolfe, A. B. MacLean, J. C. Crow, E. Benjamin, W. M. Reid and C. W. Perrett: TP53 mutations in vulval lichen sclerosus adjacent to squamous cell carcinoma of the vulva. *Br J Cancer*, 89(12), 2249-53 (2003)

87. E. Lerma, M. Esteller, J. G. Herman and J. Prat: Alterations of the p16/Rb/cyclin-D1 pathway in vulvar carcinoma, vulvar intraepithelial neoplasia, and lichen sclerosus. *Hum Pathol*, 33(11), 1120-5 (2002)

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

Send correspondence to: Anna M. Czarnecka, Laboratory of Molecular Oncology, Department of Oncology, Military Institute of Medicine, ul. Szaserow 128; 01-141 Warsaw 44, Poland, Tel: 0048226817092, Fax: 0048226103098, E-mail: aczarnecka@wim.mil.pl

http://www.bioscience.org/current/volS3.htm