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Pathogenetic mechanisms in epidermodysplasia verruciformis

ABSTRACT

Epidermodysplasia verruciformis (EV) is a rare, lifelong disease, exclusively manifested on skin. Most freguently it starts in the early childhood. Plane wart- like lesions on the limbs are combined with pityriasis versicolor-like wart on the trunk, neck and face, and reddish or brownish plaques that undergo malignant transformation in more than 60% of patients. Tumors arising are seborrhoic and actinic keratoses, Bowen´s disease and squamous cell carcinomas (SCC). Carcinomas are confined mainly to the sun exposed areas. They appear in the fourth and fifth decades of life, and develop slowly, with low invasive and metastatic potential. EV is now considered as a genetically transmitted facilitation of skin infection by one or more EV specific types of human papillomavirus (EV HPV). It raised enormous interest since it is: 1) a widespread persistent infection of the skin with EV HPVs; 2) a precancerous genodermatosis, the first model of human viral cutaneous oncogenesis; 3)characterized by long duration and slow progression, the clinical manifestations of apoptosis, which is in EV associated with inflammation; 4) a model of local defence mechanisms in the progression of HPV-associated cancers, as a consequence of apoptosis arising from immune defect in EV HPV presentation, that leads to to specific immunotolerance. The interacting immunogenetic and environmental factors, especially UV irradiation, result in the inability of patients' immune system to reject EV HPV-harboring kerationocytes. A susceptibility locus for REV has been mapped to chromosome 17 qter, in a region flanked by D17S939 and D17S802 markers. Frequences of the HLA-DRB1/11, -DQA10501, -DQB10301 haplotypes were found to be significantly higher in EV patients, and could represent susceptibility allels, or a marker in the linkage disequilibrium with a gene predisposing to EV. This could provide a genetic basis determining specific defect of cell-mediated immune responses directed against EV HPVs. Upregulation of E6 and E7 gene transcription during the EV HPV-associated malignant progression is of the basic significance. The E2 protein is the most important viral factor, regulating the expression of E6 and E7 oncogenes. Acting as a transcription factor, E2 binds specific site within the viral long control region (LCR), and this results in activation of E6 and E7 transcription and viral DNS replication. Oncoproteins E6 and E7 of "high-oncogenic" EV HPV-s may directly inhibit antioncogene p53 induced transactivation of downstream genes. This results in the aberant overexpression of non-functional wild-type p53 protein, and enhance apoptosis in EV lesion. The overproduction of TNF-alpha, and TGF-beta is one of the main mediators of UVB induced local immunosuppression in EV. However, TNF-alpha and TGF-beta are the main cytokines capable of inhibiting HPV oncogene expression at the same time. This could be one of the possible explanations for a low invasive and metastatic potential of EV cancer. In spite of heavy immunosuppression due to an immunogenetic defect, patients with EV have some important defence mechansims.

Key words: *Genodermatosis; Human papillomavirus; Viral oncogenesis; Immunity; Epidermodysplasia verruciformis*

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INTRODUCTION

Exclusively manifested on skin, with the interacting genetic, immunologic, viral factors and UV irradiation in its origin, epidermodysplasia verruciformis (EV) is a rare lifelong disease (1). Cutaneous lesions of EV are characterized by combination of extensive flat, verruca plana-like warts, with redish or brownish plaques that undergo malignant transformation in the fourth and fifth decades of life. In some, especially black patients, the lesions are highly proliferative (3).

EV has raised enormous interest since it is: 1) a widespread persistent infection of the skin with EV specific types of human papillomavirus -(EV HPV); 2) a precancerous genodermatosis, the first model of human viral cutaneous oncogenesis; 3) characterized by long duration and slow progression, the clinical manifestations of

apoptosis which is in EV associated with inflammation; 4) a model of local defense mechanisms in the progression of HPV - associated cancers, as a consequence of apoptosis arising from immune defect in EV HPV presentation, that leads to specific immunotolerance (2, 4).

EPIDEMIOLOGY

EV is an extremely rare disease but worldwide in distribution. Since 1922, when described by Lewandowsky and Lutz (5), approximately 250 cases of EV have been reported (6). It has been found in different continents and in various races. About 10% of affected families have more than one sibling affected, and 10% of affected patients are the offspring from consanguineous marriages (1,7). The proportion of EV siblings in family approaches 25% and the sex ratio for EV is close to one (1). Skin carcinomas are rare in black patients with EV and malignant transformation occurs in only 5% (8).

ETIOLOGY AND PATHOGENESIS

EV is considered as a multifactorial disease involving genetic, immunologic and environmental factors, especially UV irradiation, in addition to specific EV HPV genotypes, including oncogenic EV HPV type 5. It represents a genetically determined unusual susceptibility to infection with EV HPVs that are harmless for the general population (1,2).

Genetic background

Mode of inheritance is apparently autosomal recessive, although x-linked recessive and autosomal dominant inheritance patterns have also been describe (1).

Taking EV as a model to identify a locus underlying the susceptibility to EV HPV infection, homozigosity restricted to affected individuals was observed for markers of chromosome 17qter. The results provide significant evidence for the presence of an EV-susceptibility locus (named EV1) in a 1 centiMorgan (cM) interval flanked by D17S939 and D17S802 markers on 17qter (1).

Little is known on the host genes underlying the susceptibility to infectious diseases (9). The EV1 locus represents the first locus so far known as a gene/genes controlling the susceptibility to an oncogenic HPV genotype (1). The mutated gene/genes on EV1 could encode proteins involved in the intracellular control of infection, acting either on the expression of the viral genome or on the activity of the nonstructural viral proteins, e.g., oncoproteins. These genes could also play a part in the immunologic control of EV HPV infections (2).

It is worth stressing: 1) a dominant susceptibility locus for familial psoriasis that has been mapped to region that contains EV1, between D17S802 and D17S785 (10); and 2) a frequent loss of heterozygosity of the D17S785 marker located close to EV1 in actinic keratoses and squamous cell carcinomas of the skin (11).

It is also interesting that patients suffering from psoriasis have recently been shown to constitute the long-searched for, reservoir of EV HPV type 5. In contrast with the rare detection of this particular EV HPV genotype in skin specimens normal individuals or patients with other skin diseases (e.g., 0% in atopic dermatitis), EV HPV type 5 was detected in more than 89% of patients with psoriasis. Furthermore, antibodies against EV HPV type 5 capsid proteins were found in a significant proportion (12.5%) of psoriatic patients (12). This suggests that host restriction towards EV HPV type 5 infections is somewhat less efficient in patients suffering from psoriasis. However, patients suffering from psoriasis or EV are not known to be prone to EV or psoriasis respectively. It is tempting to speculate that distinct defects affecting the same gene/genes on chromosome 17qter may be involved in the two skin conditions (1).

The genetic nature of immunologic mechanisms controlling HPV infection is supported by findings that the immune response of T-ly against viral antigens requires their processing and presentation by proteins encoded by MHC. Thus, it is possible that the polymorphism of various genes within MHC could provide a genetic basis determining immune reactions against EV HPVs, and conditions the immune responses into specific immunotolerance (13). Recent studies have shown that specific -DR, -DQ haplotypes are associated with EV. Frequencies of the HLA-DRB1/11, -DQA10501, -DQB10301 haplotypes were found to be significantly higher in EV patients than in normal population (14). It is noteworthy that these specific HLA class II -DR, -DQ haplotypes could represent susceptibility alleles or a marker in the linkage disequilibrium with a gene predisposing to EV (2,7).

Role of human papillomaviruses

Association of EV and HPVs

The association between HPVs and disease where noted by Jablonska (15). Such relationship has been confirmed by successful auto- and heteroinoculation, and detection of viral particles in skin lesions. Since the discovery of the first EV specific HPVs (EV HPVs) in 1978 (16), over 20 cloned EV HPVs are known, and several being characterized (7).

According to recent criteria for HPV classification, a new type is recognized if it shows more than 10% dissimilarity in the nucleotide sequences of the L1 gene (17). Thus, the definition of a new EV HPV type is one, which shares less than 90% homology of its L1 open reading-frame to any other known HPV (6). Aminoacid or nucleotide sequences underline phylogenetic or evolutionary trees, and reflect tissue tropism and oncogenic potential of HPVs. This is the basis for classification of HPVs into several groups: cutaneous; cutaneous involved in EV; cutaneous and mucosal; and mucosal of low and high risk (e.g., without or with oncogenic potential) (18). Althouhgh the phylogenetic classification is still not applicable for clinical purposes, EV HPVs constitute a separate group, with only HPV 48 and 50 as distantly related types (Table 1,2) (17).

Table 1. Cutaneous HPVs infections

HPV type	Lesions
1	Myrmecia wart (verruca plantaris)
2	Common wart
3	Plane wart (verruca plana)
4	Common wart
7	Butcher's wart (proliferative hand warts)
10	Plane or intermediate wart
26,27	Intermediate wart (mostly in immunosuppressed)
28	Plane or intermediate wart
29	Intermediate or common wart
34	Bowen's disease (but mainly genital)
36	Actinic keratosis (mainly EV HPV)
37	Keratoacanthoma (single case)
38	Malignant melanoma (single case)
41	Warts, squamous cell carcinoma
48	Squamous cell carcinoma (immunosuppression)
49,50	Warts, premalignant lesions (immunosuppression), recognized as EV HPV
57	Inverted maxillar papilloma (mainly genital)
60	Epidermoid plantar cyst
63	Myrmecia cystic wart
65	Pigmented wart
75,76,77	Common wart (immunosuppression)

HPV= human papillomavirus
EV= epidermodysplasia verruciformis

Table 2. EV-specific HPVs

HPV type	Lesions
5,8,20,47	Benign lesions
	EV cancer
9,12,14,15,17,19,22,23,24,25,36,46,59	Benign lesions
49	Plane warts

HPV= human papillomavirus
EV= epidermodysplasia verruciformis

There is a great diversity of EV HPVs and almost no homology between EV HPVs and other HPVs. The heterogeneity can perhaps be explained by millions of years of HPV evolution that prompted its adaptation to various host cells and changes in host control of infection (17). It has been revealed that the major evolutionary separation between genital (mucosal) papillomaviruses (PVs) and EV HPVs, hitherto found only in HPVs, also exists in animal PVs (e.g., Abyssinian Colobus monkey). The presence of these two major phylogenetic divisions of PV in both human and monkey hosts, strongly suggests that this diversification predated the evolutionary split between monkeys and apes. In other words, at least two different groups of PVs have been evolving separately in their respective primate hosts for more than 22 million years with only moderate sequence changes since their genesis (19).

Based on DNA homology, EV HPVs could be classified into four groups: 1) HPV types 5,8,12,14,19-23,25,26; 2) HPV types 9,15,17,38,47;

3) HPV 24; 4) HPV types 3,10 (13). HPV types 3,10 are responsible for plane and intermediate warts in the general population. Thus they are not EV specific HPVs, but they are sometimes present in patients with EV, more often as a mixed infection. Another classification divides EV HPVs into the group with high-oncogenic potential (HPV types 5,8,47) which appear in more than 90% of the EV skin carcinomas, and the group with low-oncogenic potential (e.g., HPV types 14,20,21 and 25), which are usually detected in the benign EV lesions (13).

EV is now considered as a genetically transmitted facilitation of skin infection by one or more EV specific HPV types. The benign lesions are associated with diverse EV HPVs. In contrast with the plurality in the benign tumors, only few types, predominantly HPV types 5 and 8, much less frequently HPV types 14,17,20 and 47 can be detected in EV carcinomas (7).

The frequent detection of EV HPVs in nonmelanoma skin cancers both in immunosuppressed (90%) (20), and immunocompetent populations (50%) (21), supports the importance of EV as a model of cutaneous oncogenesis. In NMSC occurring in organ transplant recipients, the most prevalent were EV HPVs types 15,19,20,23-25 and 38 (20,21). In an immunocompetent population, of 4 EV HPV types disclosed, one was potentially oncogenic, HPV type 8, known to be associated with tumors in EV (21). In addition to EV HPV DNA sequences, a great number of other cutaneous and mucosal HPVs were found in the cancers of immunosuppressed and immunocompetent patients. This is in contrast to EV carcinomas that are never associated with mucosal HPVs, and harbor, as a rule, only oncogenic EV HPVs (2).

Because the heavy immunosuppression is associated with all types of HPVs among others EV HPVs, it is conceivable that EV HPVs are widely distributed, and are present in latent form in the general population (2). The presence of EV HPV-DNA in plucked hairs from all renal transplant recipients and in the considerable proportion (45%) of hairs from healthy volunteers (22), as well as the demonstration of EV HPVs in 35% of normal skin biopsies (20), indicate that EV HPVs are subclinically present in the skin of the general population, and could be activated by immunosuppression (7). Thus immunosuppressed population may be regarded as a reservoir of overt or latent EV HPVs (2).

EV HPVs are not pathogenic but harmless for the general population. Infection associated with EV HPVs occurs in heavily immunosuppressed patients such as renal alograft recipients, patients with Hodgkin's disease, systemic lupus erythematosus or malignant neoplasms associated with severe immunodeficiency, and more recently in patients infected with HIV. Most of the lesions failed to show the morphology of typical EV lesions, or the characteristic cytopathic effect that is associated with EV HPVs (7).

In immunosuppressed patients with preserved host-cell restriction of the EV HPV genome, infection with EV HPVs has no typical morphology and is rather transient. In these individuals EV HPVs may also be codetected in plane warts induced by HPV types 3,10 and 28, that have characteristics of warts in the general population (2).

However, the incidence of EV during HIV infection is very low, suggesting the intervention of genetic background (7). Moreover, it seems that the mere presence of EV HPVs appears not to be sufficient for a complex expression of the EV phenotype, since EV HPV-DNA has been detected in typical cutaneous warts with no feature of EV, whereas EV changes develop only in a small number of immunosuppressed patients who are more susceptible to breakdown of a host-cell restriction, because of genetic alternations (2).

Epidermodysplasia verruciformis-specific HPVs mostly types 5 and 8 were repeatedly found in patients with renal alografts in lesions having clinical and histological features of EV. Showing clinical similarity to true EV, this so-called EV-like syndrome can be distinguished by the later onset, less widespread cutaneous lesions and the presence of other symptoms of immunosuppression (13).

Structure of the HPV genome

The HPV genome consists of approximately 8000 base pairs (bp) double-stranded, circular DNA that encode 9-10 open reading frames. It could be divided into parts labelled as "L" (late), "E" (early) and "LCR" (long control region) (Fig.1). The L1 and L2 genes encode viral capsid proteins. The "E" region consists of genes involved in regulation of HPV-DNA replication (E1 and E2) or cell proliferation and immortalization (E6 and E7). The E2 protein serves as in important transcription factor regulating the expression of E6/E7 oncogenes. The LCR contains cis-active elements as highly specific receptors that bind various transcriptional factors involved in the regulation of both viral DNA replication and gene expression (17).

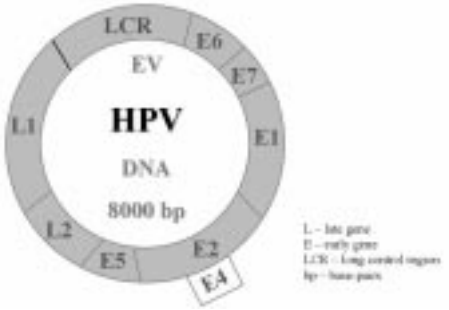

Figure 1. Scheme of genome organization of HPV

EV HPV associated oncogenesis

The two proteins of high-risk HPVs E6 and E7, have oncogenic potential in vitro, and can interfere with cellular factors involved in the control of cellular proliferation and in the prevention of cell immortalization. Up regulation of E6 and E7 gene transcription during a multistage-process of EV HPV-associated malignant progression is of the basic significance. The E2 protein is the most important viral factor regulating the expression of E6 and E7 oncogenes. Acting as a transcription factor, E2 binds specific site within the LCR and this results in activation of E6 and E7 transcription by stimulating the promoters of these gene (17).

Oncogenes E6 and E7 are constantly expressed in cancer cells. The regulatory mechanisms of E6/E7 expression are absent in cancer cells. Oncoproteins E6/E7 are capable of immortalizing cells, inducing cell growth, and promoting chromosomal instability in the host cell (17).

Thus, in anogenital cancers associated with HPV 16 and 18 types, E6/E7 viral oncoproteins inactivate and degradate antioncogene (tumor suppressor) protein p53 and retinoblastoma susceptibility protein (pRB) by complex formation. The conversion of tumor suppressor gene p53 into oncogene appears to be of special importance for tumors associated with genital "high-risk" HPVs (17). The "high-risk" E6 oncoprotein interacts with the product of antioncogene p53, leading to degradation of p53 protein by cellular ubiquity proteolysis system. The p53 protein acts normally as cell-cycle checkpoint, and the p53 pathway seems to play an important role in maintainance of the genetic integrity. In response to DNA damaging influences, such as UV light, normal (wild-type) p53 accumulates in cell nuclei causing cell-cycle arrest at the G1 phase. This process allows the cell to repair DNA before further cell division. In same cells the G1 arrest induces apoptosis (23). Because E6 and p53 interaction leads to unblocking of cell division and host DNA synthesis, it is presumed that this interaction is the main cause of chromosomal instability, and accumulation of various mutations in cell infected with genital "high-risk" HPVs (24). On the other hand, deletion, mutations and functional inactivation of tumor suppressor genes may be a key event in tumor genesis (23). Moreover, the E7 oncoprotein of "high-risk" HPVs forms a complex with pRB, with much higher affinity than E7 of "low-risk" genital HPVs. The complex formation leads to release of transcription factor normally bound to pRB, that is the E2 protein, which activates transcription of variety of genes involved in regulation of cell proliferation (17).

Yet, though it is conceivable that the inactivation of an unknown tumor suppressor gene and/or activation of a cellular oncogene play a role in EV pathogenesis and in the development of EV cancers, the mechanism of action of E6 and E7 oncoproteins of the oncogenic ("high-oncogenic" EV HPV types 5,8,47) appears to differ considerably from those described for E6/E7 of "high-risk" anogenital HPVs (2,6,13,17,23). It seems that characterization of genetic and chemical differences between "high-oncogenic" and "low-oncogenic" EV HPVs could also be very helpful in understanding the process of viral cutaneous oncogenesis, but the characteristic

features of EV HPVs associated oncogenesis are: 1) a frequent expression of E6 and E7 oncoproteins in the cutaneous tumors, with a very rare, only in metastatic tumors, integration of viral DNA into the host-genome; 2) inability of E6 protein of oncogenic EV HPVs to degradate p53; 3) lack of transforming activity of E7 protein of oncogenic EV HPVs; 4) unusual, as compared to other types of HPVs, genomic heterogeneity of E6 and L1 proteins of oncogenic EV HPVs, which might reflect immunologically selective mechanisms related to formation of antibodies against these proteins; and 5) rare mutation of p53 in EV tumors in contrast to skin cancers in the general population (2,23). Moreover, in a majority of EV cancers, Majewski et al found the accumulation of a wild form of p53, up regulation of bax protein expression and lack of bcl-2 protein (2). It seems that this pattern of p53, bax and bcl-2 expression could facilitate and enhance apoptosis, especially in the sun exposed skin areas. This assumption is supported by highly characteristic ultra structural features of apoptosis in EV (15).

Pizarro et al. investigated p53 expression in a series of viral warts from patients with EV. Immunostaining was positive in 92% warts, but negative in most cases of warts from non-EV patients. Mutations of the p53 gene at exons 5-8 were not detected, suggesting an association between p53 accumulation probably of wild-type and EV warts (23). An important question raised by these results is the functional status of the p53 protein accumulated in benign EV lesions. Over expressed p53 protein in EV warts could be: 1) accumulated functional wild-type p53 protein in response to cellular stress induced by EV HPV infection; 2) aberrant accumulation of non-functional wild-type p53 protein, because of its interaction with specific viral proteins or cellular proteins which could be expressed in response to EV HPV-type infection (23).

Furthermore, the predisposition to skin cancers in EV patients supports the idea that over expressed p53 protein in EV warts might not be functional, or its suppressor function may be overcome by the unknown mechanism leading to malignant transformation.

Pizzarro et al also demonstrated convincing p53 immunostaining in suprabasal keratinocytes in lesions showing typical EV-like cytopathic changes but no atypia. This is particularly striking because McGregor et al did not find this pattern of p53 immunostaining in benign virus warts from renal transplant recipients who are also predisposed for EV HPV infection (25). These observations suggest that both cytopathic effects and p53 accumulation seen in EV-associated virus warts may reflect host-related factors as well (25).

It is of interest that aberrant over expression of non-functional wild-type p53 protein has been observed in both cancer cell lines and cancer-prone patients (26). The fact that no complex formation could be detected between the EV HPV E6 protein and the cellular p53 does not exclude the other mechanism by which EV HPV infection could affect p53 levels, conformation or function (23). Oncogenic EV HPV types 5 and 8 do not appear to associate with pRB or target p53 for degradation, but preliminary studies show that the E6 and E7 proteins of EV HPV type 5 and 8 may directly inhibit p53 transactivation of downstream genes (6). This may be important for their oncogenic activity (Figure 2), but it seems likely that other, as yet unidentified cellular targets, also exist (6,23).

Figure 2. Highly specific cis-receptors for various transcription factors

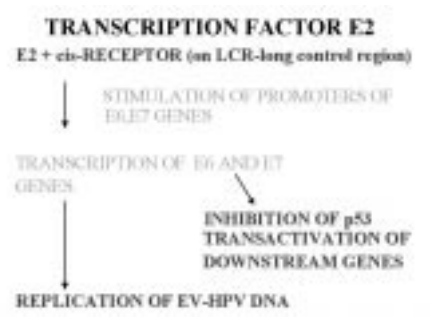

Figure 3. EV associated oncogenesis

Although further studies are needed in order to understand effects of the EV oncoproteins on p53, one of the possible factors that could facilitate cell transformation by EV HPV genes is, as it was previously mentioned, E2-coded transcription factor. It acts as a trans-activator of regulatory elements within the LCR of oncogenic EV HPVs. It was shown that the loss of E2 transactivating function is associated with the loss of transforming effect of this gene. In addition to transcription factors both viral and cellular, several cis-active transcription control elements have been identified within the LCR of the viral DNA. Some of these sequences are negatively acting. Since LCR contains sequences that bind the viral trans-activator E2, it was suggested that viral factor E2 could relieve the repression resulting in enhancement of E6/E7 transcription during the early stages of malignant progression (13). Moreover, it was shown that sequences surrounding transcription start sites of the EV HPV type 8 oncogene E6, and comprising E6 promotor, contain three elements similar to the DNA recognition site of the multifunctional cellular transcription factor ying-yang (YY1). It was demonstrated that these sequences indeed act as YY1 binding sites. With regard to their functional relevance for E6 promotor activity, it was shown that YY1 turned out to exert both positive and negative effects, depending on which sequence binds. Thus binding of YY1 to a site upstream of the promotor´s (BS1 sequence) activated transcription of E6 oncogene, whereas the downstream site (BS2 sequence) mediated regression. The second downstream YY1 binding site (BS3 sequence) seemed to play an auxiliary role. These observations confirmed YY1 transcription factor as an important cellular protein involved in control of E6 oncogene expression of the "high-oncogenic" EV HPV 8, and emphasized the potential regulatory role of cis-active elements, acting as highly specific receptors (27).

Role of cellular mechanisms regulating EV HPV oncogene expression

It is well established that many EV lesions show slow progression. Thus, EV cancers are characterized, in spite of histological malignancy, by a very low metastatic potential. This is due to an intracellular control, the presence of antioncogenes and other cellular mechanisms that suppress viral gene transcription and the function of viral oncoproteins (17).

Intracellular signal mediators

It is evident that in addition to the presence of E6/E7 oncogenes, some host-DNA modifications are a prerequisite for cell imortalization and transformation (24). Unfortunately, the nature of putative cellular suppressor genes involved in the control of EV HPV oncogene expression and prevention of cell imortalization are to be elucidated.

Transcription factors regulating promoter-enhancer region

Numerous cellular transcription factors that constitute a network within the cell, regulate transcription of E6/E7 oncogenes. Some are capable of either stimulating (e.g., AP-1, SP-1 factors) or inhibiting it (e.g. Oct-1, YY-1, and retinoic acid nuclear receptors-RAR) (17). As it was previously shown for YY-1, some factors express both positive and negative effects (27).

Of special importance is the absence of RAR-beta in malignant cells. These results suggest that some dysregulation of RARs could be important for the tumor progression in HPV-associated tumors (28).

Cytokines and other biologic modifiers

The second line of defence includes the role of cytokines and adhesive molecules in the growth of EV tumors.

Cytokines exert various autocrine and paracrine effects. Therefore they could control EV HPV infection as well as oncogenesis, not only by inhibiting oncogene expression but also by modifying other steps, such as inhibition of metastasis (2,17).

The main cytokines capable of inhibiting HPV oncogene expression include TNF-alpha, IL-1, IL-6, TGF-beta, IFN-gamma, leukoregulin, and EGF (17).

TGF-beta is major factor regulating synthesis of the components of extracellular matrix (ECM) and the expression of the ECM receptors, for example, VLA integrins. Majewski et al. studied the expression of various integrins : VLA-2 (collagen receptor), VLA-2 (collagen receptor), VLA-3 (multispecific receptor for collagen, laminin and fibronectin), and VLA-6 (laminin receptor) in EV skin lesions, as compared to that in benign, premalignant and malignant lesion in the general population. They found a simultaneous increase of VLA-2 and VLA-6 in EV lesions associated with EV HPV types 5 and 8, but not with HPV 3. An increased expression of VLA 6 was found especially in the areas with an increased TGF-beta expression (2,13). It seems that increased VLA-6 expression that is associated with TGF-beta-dependent down regulation of the activity of ECM-degrading enzymes, could contribute to a low invasiveness of EV tumors. Thus, the anti-invasive effects of TGF-beta could be attributed to its capability to: 1) inhibit tumor angiogenesis; 2) stimulate synthesis to the components of ECM; 3) induce the tissue inhibitor of metalproteinases; 4) down regulate the activity of ECM degrading enzymes; 5) stimulate the expression of VLA-6 (2,17).

Role of defective immune surveillance in EV

The most characteristic immunologic feature of EV is a defect of cellular immunosurveillance mechanisms, leading to their inability to eliminate EV HPV-harboring keratinocytes. Because non-specific systemic immunity is only partially defective in EV patients and the patients are not prone to infection with non-EV HPVs (e.g., genital HPVs), it seems that the lack of response to EV HPVs is either selective or restricted to antigens presented locally in the skin-associated lymphoid tissue (2). The nature of this defect is unknown. However, the existence of familial cases and a very low incidence of EV in heavily immunosuppressed patients (e.g., during the HIV infection), strongly suggest its genetic background (7).

Variable abnormalities of non-specific cell-mediated immunity, both local and systemic, were found in patients with EV. Yet, though a decreased absolute number of T-ly and helper T-cells with a reversal of the T-CD4+/T-CD8+ cell ratio, and an expansion of CD8+ large granular lymphocytes has been described in EV, one of the most important and constant abnormalities is the cutaneous anergy to locally applied contact sensitizers (e.g., dinitrochlorobenzene) (29). No significant changes in the number and morphology of Langerhans cells were found in patients with EV (30,31). An existence of genetically determined defective function of these cells appears possible, leading to an abnormal presentation and/or recognition of EV HPV antigens (2,13).

Specific defect of cell-mediated immunity

A highly characterized defect of cell-mediated immunity in EV is the pronounced inhibition of natural killer (NK) cell-mediated, and T-cell-mediated cytotoxicity against EV HPV-harboring keratinocytes (specific target cells). In parallel, the immune-cytotoxic mechanism against non-EV HPV target cells is well preserved (30,32). On the other side, in patients suffering from EV, T-ly with gamma/delta receptors, characterized by specific immunotolerance towards EV HPVs and with preserved immune reactivity against other pathogens were found in great number (2,33).

Role of ultraviolet radiation in abrogation of local immune surveillance

The malignancies develop mostly in sun-exposed areas. Sunlight, in conjunction with immunogenetic factors and EV HPVs may substantially affect the process of carcinogenesis. UV radiation is a main environmental factor, evoking not only DNA mutations (e.g., of p53 antioncogene), but also the local-cutaneous, or systemic immunosuppression. On the other side, lymphocytes from EV patients have an abnormal repair response to UV light-induced DNA damage, resulting in a high frequency of radiation-induced chromosome, particularly chromatid-type aberrations. This abnormality is probably caused by deficiency in DNA repair, and it is consistent with the patients' sensitivity to UV light-induced cutaneous squamous cell carcinomas (SCC). In contrast, gamma-ray challenge produced normal repair response (34,35).

Instead of Thl-type reactivity, after irradiation with UVB rays, Langerhans cells (LC) from EV patients preferentially induce Th2-type immune reactions. These could be due to UVB-dependent decrease in the expression of ICAM-1, that is necessary for LC and T-ly cooperation (36). However, UV radiation induces derangement of viral and host interplay in keratinocytes, and this is probably the most important factor in EV oncogenesis. Thus, an increased expression of ICAM-1 on "non-professional" antigen-presenting keratinocytes, (found in EV lesions) could favor presentation of EV HPV antigens in a tolerogenic manner (2).

UVB-inducible cytokines TNF-alpha, TGF-beta, IL-10 and aMSH, are of crucial importance for the inhibition of the process of antigen presentation. Two of these cytokines, TNF-alpha and TGF-beta, were found to be over expressed in lesional epidermis of EV patients (2).

Over expression of TNF-alpha could contribute to the induction of local immunosuppression or immunotolerance towards EV HPV-harboring cells, because this cytokine was shown to prevent Langerhans cells from migrating into the regional lymph nodes. Up regulation of TNF-alpha expression seems to be due to an increased production of the cis-isomer of urocanic acid (cis-UCA) after the UV exposure (37). It was shown that stratum corneum of the epidermis from sun-exposed areas in EV patients contains several-fold increased levels of the cis-isomer (37). Family members of EV patients also showed increased levels of this isomer in the stratum corneum. The exact mechanism of the induction of immunotolerance by cis-UCA is not known, but it is highly suggestive that TNF-alpha could be a main mediator of this effect (2,13).

Another cytokine found to be over expressed in EV lesions, TGF-beta, might play role in induction of local immunotolerance towards EV HPV. Various paracrine effects of TGF-beta include: 1) inhibition of T-cell proliferation; 2) decrease of IL-1 dependent antigen presentation; 3) inhibition of natural cytotoxicity; and 4) suppression of lymphokine-activated killer cell cytotoxicity (38).

Thus, the over expression of TNF-alpha and TGF-beta is one of the main mediators of UVB induced local immunosuppression in EV. However, TNF-alpha and TGF-beta are the main cytokines capable of inhibiting HPV oncogene expression at the same time. This could be one of the possible explanations for a low invasive and metastatic potential of EV cancers.

CLINICAL CRITERIA

The recognition of EV is based on:

- Onset in the early childhood;
- In some cases, familial occurrence and/or consanguinity of the parents;
- Characteristic cutaneous changes, such as plane wart- or pityriasis versicolor-like lesions, and red or brownish plaques, irregular or polycyclic in shape;
- Localization of the plane wart-like lesions on the dorsa of the hands and on the limbs, and of the pityriasis versicolor-like changes, red and brownish plaques, on the neck and trunk, often also around the pubical region;
- Persistence of cutaneous lesions and their slow progression;
- No involvement of mucous membranes and lymph nodes;
- Good general condition without subjective complaints;
- Malignant conversion of some lesions, mostly in the fourth and fifth decade of life, mainly on sun-exposed areas on the face, but also in the traumatized areas (2,13,17).

PATHOHISTOLOGY

Histology of benign lesions

The most characteristic microscopic feature of benign EV lesions is a specific cytopathic effect (CPE). This effect is identical for all EV HPVs, and its intensity depends only on the viral load and disease activity. Large, dysplastic cells, with almost homogenous clear cytoplasm

and small pyknotic or vacuolized nuclei appear in nests, starting suprabasally and frequently replacing almost entire epidermis. Prominent, ovoid, round, or irregular keratohyaline granules of various sizes in the clear dysplastic cells of stratum granulosum are highly characteristic, and of diagnostic significance. The horny layer is loose and has a basket weave-like appearance with parakeratosis (2,13).

Electron microscopy of dysplastic cells reveals viral particles in the nuclei and nucleoli, clearance of nucleoplasm and cytoplasm, and prominent irregular and scattered keratohyaline granules (2,13).

Histology of premalignant and malignant lesions

The histologic appearance of premalignant lesions in EV has a pattern similar to that of actinic keratosis, with more numerous dyskeratotic cells and pronounced Bowen's atypia. Frequently, basaloid features could be seen in the lower layers. CPE disappears, or is visible only in the neighboring epidermis with no signs of malignant transformation (2).

Malignant conversion starts as a downward proliferation of the epidermal rete ridges, and around hair follicles. EV carcinomas are of squamous-cell type, with features of Bowen's atypia: multinucleated and dyskeratotic cells, with large pleomorphic hyperchromatic irregular nuclei, and numerous, frequently atypical mitoses. Some carcinomas in EV are typical basaliomas. In some carcinomas, it is possible to trace the origin of the basaloid differentation from actinic keratosis with features of Bowen´s atypia. A ratio of 16 SCCs to 1 BCC was estimated among EV patients (6).

Electron microscopy of the malignant tumors has all ultra structural characteristics of Bowen´s atypia, that is dyskeratosis and apoptosis.

The apoptotic bodies are surrounded by a single or a double membrane, and engulfed by neighboring keratinocytes. They are composed of compact bundles of tonofilaments intermingled with remnants of mitochondria and endoplasmic reticulum (39).

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of EV is based on: 1) clinical criteria; 2) pathohistology; and 3) detection of EV HPVs in EV lesions (2).

The most suitable method for identification of HPV types and subtypes, is nested, two step PCR, with the use of consensus primers targeting L1 region, and degenerate or semidegenerate primers, with repeated amplifications in the second step. Southern blot and dot blot hybridization allow estimation of the amount of viral DNA in tissue. In situ hybridization remains the best method for evaluating the tissue distribution of HPV-DNA, after HPV type has been identified (17,40).

Differential diagnosis with plane warts, lichen planus and pityriasis versicolor is based on typical diagnostic criteria for EV. Differentiation of true EV from EV-like syndrome is based on the fact that EV-like syndrome has later onset, with less widespread cutaneous lesions, and presence of other symptoms and signs of immunosuppression. Acrokeratosis verruciformis differs in its more restricted verruca plana-like lesions, localized mainly on the hands and feet, the stationary course, dominant mode of inheritance, and the absence of cytopathic effect, characteristic for EV HPVs (2).

THERAPY

Presently, there is no specific therapy for HPV infection.

The inhibition of virus replication should prevent tumor development, and this is the aim of vaccination with the use of virus-like particles (VLPs). VLPs from EV HPV type 5 variants has been prepared, and used for serologic enzime-linked immunosorbent assay (ELISA) to find the humorel response to HPV type 5 in EV patients as compared to the general population (2).

Neutralizing antibodies generated after immunization with VLPs are predominantly type specific. In future, VLPs of HPV 5 could be applied for immunoprofylaxis. For therapeutic purposes the induction of neutralizing antibodies is not sufficient, because cellular responses appear to be of prime significance. No vaccination is available either for EV patients or for prevention of the disease. However, it is not known whether vaccination will eradicate the latent infection, or only the cutaneous lesions, and how long-lasting the effect will be (2,17).

Application of retinoids in EV produced some improvement, but in general, in all cases, the results were transitory and not satisfactory. Combined therapy with retinoids and IFN-alpha was found to have a synergistic antiproliferative and antiangiogenic effect on HPV-harboring cells lines.

Thus, therapy of EV lesions includes:

1. Isotretinoin 1mg/kg body weight per day for benign lesions;

2. Combined therapy of isotretinoin (0,4 mg/kg body weight daily), 1,25-dihydroxyvitamin D3 (0,5-1,0 microgram/daily) and IL-12 (in small doses) for prevention of malignant conversion;

3. IFN-alpha 2 (1,5-6 million units daily) in combination with isotretinoin (1 mg/kg body weight daily), and IFN-alpha 2 (1 million units 3 times weekly for three weeks) intralesionally, 5 FU (5% ointment) or retinoic acid (0,05-0,1% cream) topically, for EV carcinomas (2).

The best profylaxis is removal of the whole frontal skin with the numerous lesions, and its replacement with a cutaneous graft from the internal sites of the arm. This prevented the development of cancer for up to 20 years (41).

Mizutani et al, reported a new surgical treatment for cancer lesions in patients with epidermodysplasia verruciformis, by means of cultured epidermal sheet grafts (42). Their argument for this approach is the ability to exclude HPV infection in cultured epidermal sheets.

To treat the lesions with virus-free keratinocyte cultures seems ideal only at the first sight. The conventional treatment with split-thickness skin grafts showed that unaffected grafts, after transplantation, will soon become infected with HPV (41). Such infection is likely even in cultured keratinocytes, but the development of a reccurent cancer lesions will need again a lenghty period of co-factor exposure. It is a matter of time and not of virus purified cultures. The only advantage of cultured sheet grafts might be the banking of the cultured sheet grafts (43).V

COURSE AND PROGNOSIS

Multiple cancerous lesions develop after middle age. The number of cancerous lesions increases annually. The 60% incidence of malignancies in EV patients appears to be underestimated since the long, over 30-35 years follow up of EV cohort, showed carcinomas developing in all the patients (2).

CONCLUSION

Epidermodysplasia verruciformis is a model not only of cutaneous viral oncogenesis, but also of local defense in the progression of HPV-associated cancers. In spite of heavy immunosuppression due to an immunogenetic defect, patients with EV have some important defense mechanisms.

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