Egyptian Dermatology Online Journal
Volume 2 Number 2

Study of Survivin in Verruca Vulgaris and Condyloma Acuminata: Correlation with other proliferation and apoptosis markers.

Ahmad I. Rasheed - Zeinab A-R. Kamar*

Egyptian Dermatology Online Journal 2 (2): 4, December 2006.

* Department of Dermatology & Venereology - Ain-Shams University

*Department of Pathology - Ain-Shams University

Accepted for publication in: June, 2006.

Abstract

Background: HPV-related lesions represent an attractive model to study the interaction between proliferation and apoptosis regulatory factors that ultimately control cell dynamics and determine tissue mass.

Aim of the work: was to study the expression pattern of some proliferation and apoptosis regulatory proteins in two of the HPV-related lesions namely verruca vulgaris and condyloma acuminata.

Material and methods: Eleven lesions of verruca vulgaris, eleven lesions of condyloma acuminata and eight normal skin specimens were examined immunohistochemically for reactivity for survivin, p-53, Bcl-2 and PCNA.

Results: Markedly increased staining for survivin and p-53 was observed in both VV and CA in comparison to normal skin. PCNA expression was also enhanced in both types of viral lesions but more in VV. Bcl-2 expression was very faint in both VV and CA. Strong positive correlation could be seen between survivin and p-53 scores of reactivity.

Conclusion: Both enhanced proliferation and decreased apoptosis seem to play some role in the evolution of HPV-related neoplasms. A major role for survivin can be postulated while its

significance in relation to malignancy seems to be doubtful. A possible role for p-53 in restoring the balance to the dynamics of virally infected cells can be suggested

Introduction

Tissue homeostasis requires a tightly regulated program of cell division and cell death. Apoptosis, or programmed cell death, counters proliferation and is regulated by an intricate system of pro-apoptotic and anti-apoptotic molecules.[1]

Dysregulation of apoptosis is a common feature of cancer[2]and a likely factor in the development of both benign and malignant cutaneous neoplasms.[3]

For many reasons, the HPV-induced skin lesions (the so-called verruca) represent an attractive field for studying proliferative and apoptotic control of neoplasms. The relation of these hyperplastic, relatively non inflammatory lesions to the specific viral aetiology is well documented and exhaustingly classified[4], the genetic aspects of viral replication and their influence on the host cell machinery are well studied[5], the viral papillomas represent a striking example of somewhat rapidly evolving hyperplastic lesions, the incidence of the infection is relatively high[6]and their cutaneous and superficial location makes the lesions easily accessible for clinical follow up and for biopsy. Lastly, the diversity of the clinical spectrum corresponding to different serotypes[4], as well as the propensity of some variants for malignant transformation[7], make them invaluable for the purpose of analytic studies.

Both apoptosis and proliferation seem to be regulated by complex interplay of numerous factors. Among these, survivin stands as one factor that has gained much interest in the last few years.[8]

Survivin is an IAP family member that has been implicated as a regulator of cell division as well as an inhibitor of apoptosis. Survivin associates with the mitotic spindle in the G2/M phase of the cell cycle and is essential for proper execution of mitosis and cell division.[9]Its specific expression in G2/M is transcriptionally controlled,[10]as is typical of mitotic genes.[11]Interference with survivin-microtubule interaction results in a failure of the antiapoptotic function of survivin and an increase in caspase-3 activity with subsequent apoptosis.[12]Disruption of survivin function has likewise been linked with cell division defects.[8]

Unlike other IAP proteins, survivin appears to function more as a regulator of mitochondrial apoptosis rather than as a caspase inhibitor. However, the precise mechanism by which survivin suppresses apoptosis is still incompletely understood. Several mechanisms are theorized which include direct suppression of

caspase-3,[13]binding to caspase-9,[14]Hepatitis B X-interacting protein-mediated binding to procaspase-9, thus preventing apoptosis via the intrinsic pathway,[15]and indirect inhibition of caspases through interaction with intermediate proteins e.g. By binding the proapoptotic protein Smac/DIABLO.[16]The antiapoptotic cytoprotection offered by survivin is ineffective against the Fas-induced apoptotic pathway.[8]

Survivin is expressed during embryonal development but is almost absent in most normal, terminally differentiated tissues. Normal adult human tissues that express survivin include thymus,[17]basal colonic epithelium,[18]gastric mucosa,[19]and CD34+ hematopoietic stem cells. In contrast to its limited distribution in normal, terminally differentiated tissues, survivin expression is significantly upregulated in many human malignant tumors where its expression seems to be associated with a more aggressive phenotype, shorter survival times, and a decreased response to chemotherapy.[8]

Survivin expression has been also associated with viral-induced neoplasms where it is again suggested as an early indicator of malignancy.[20]

It is not known why this protein is upregulated in cancer. Multiple tumorigenic pathways seem to be potentially involved. Wild-type p53 transcriptionally represses survivin, whereas mutant p53 (associated with many malignancies) may contribute to upregulation of survivin. Constitutive STAT3 activation and upregulation by c-H-Ras oncoprotein may also result in survivin over-expression. As regards non-transformed cell lines, upregulation of survivin in endothelial cells is observed after stimulation with VEGF, basic fibroblast growth factor, and angiopoietin-1. In CD34+ hematopoietic stem cells, there is upregulation of survivin after incubation with a combination of thrombopoietin, Flt3 ligand, and stem cell factor.[8]

As regards the skin, survivin has been also shown to be almost absent under normal conditions. Its expression was found to be upregulated in melanoma, non-melanoma skin cancers, benign melanocytic nevi, and some benign keratinocytic neoplasms.[21], [22]

P53 is another regulatory protein that is encoded by one of the tumor suppressor genes and is implicated in normal tissue homeostasis as well as in the pathogenesis of many neoplastic disorders. It contributes to several cellular activities including apoptosis, transient growth arrest, and sustained growth arrest (or senescence). It plays a major role in the cellular response to DNA damage and other genomic aberrations. In normal cells, p53 is present in a latent form and its levels are very low as the MDM2 protein binds to p53 and increases its susceptibility to proteolysis by the 26S

proteosome. In a cell that undergoes stress, p53 can be activated by phosphorylation at multiple sites e.g. by ATM, ATR, and DNA-PK. This phosphorylation impairs the ability of MDM2 to bind p53, promoting both the accumulation and functional activation of p53. In response, the cell may undergo cell-cycle arrest at G1 checkpoint (to allow for DNA repair before replication) or undergo apoptosis (if DNA damage is beyond repair)[23]

The active p53 would induce cell cycle arrest or apoptosis through transcriptional regulation of several genes including the cell cycle inhibitor p21, DNA repair gene GADD45, and the apoptotic inducer Bax. p53 can also initiate apoptosis through a direct signaling pathway.[24]

Besides MDM2 inactivation under normal conditions, p53 can be functionally inactivated under pathologic conditions e.g. by mutation or binding to DNA tumor virus encoded proteins, such as SV40 large T antigen, Adenovirus E1B and papilloma virus E6 proteins. If p53 function is lost from any cell, the ability to eliminate potentially cancerous cells is lost and cancers can form. In fact, p53 has been found to be frequently mutated in the majority of human cancers.[25], [26], [27], [28], [29], [30], [31]

PCNA is a marker for cells in early G1 phase and S phase of the cell cycle. It is found in the nucleus and is a cofactor of DNA polymerase delta. It helps increase the processivity of leading strand synthesis during DNA replication. In response to DNA damage, PCNA is ubiquitinated and is involved in the DNA repair pathway.[32]

Bcl-2 is a proto-oncogene found on the mitochondrial membrane and acts to protect the cell from undergoing cell death.[33]It acts through two mechanisms: firstly, it prevents increased mitochondrial permeability thus blocking cytochrome c release from the mitochondrial membrane. Secondly, it acts as a "docking protein" to bind and sequester APAF-1 in order to prevent the activation of caspase 9.[34]

Within the epidermis, Bcl-2 is found in abundance only in proliferating basal keratinocytes, thus making it an important inhibitor of basal cell apoptosis.[35], [36]There seems to be loss of Bcl-2 expression as epidermal cells migrate upward, thus making suprabasal cells more susceptible to apoptosis.[33]Furthermore, it has been proposed that the loss of Bcl-2 may even be a signal for upward migration of cells within the epidermis.[35]

Aim of the work

The goal of the present study was to determine the expression pattern of survivin in two types of HPV- induced lesions namely

verruca vulgaris (as an example of totally benign neoplasms) and condyloma acuminata (as an example of benign neoplasm with occasional oncogenic potential).[37]The study also aims to correlate survivin expression with that of some other apoptosis and proliferation markers namely p53, Bcl-2, and PCNA.

Material and methods

Eleven clinically typical lesions of verruca vulgaris (from 8 patients) and 11 typical lesions of condyloma acuminata (from 7 patients) have been excised under local anesthesia and immediately fixed in 10% formalin for subsequent embedding in paraffin blocks. Each biopsy was cut into 4 um-sections (10 were prepared for each specimen). One section was stained with hematoxylin and eosin (H&E) for routine histopathologic examination (to confirm the diagnosis and to record relevant histopathologic features) while other sections were subjected to immunohistochemical staining where 4 sections from each biopsy have been stained for each of the 4 markers under evaluation (namely survivin, p-53, Bcl-2 and PCNA).

Excluded from the study were clinically or histopathologically atypical lesions, lesions showing evidence of inflammation or trauma, cases with previous trials of treatment, patients with major medical illness or associated skin disease and cases below the age of 10 years or above the age of 50 years.

Eight normal skin specimens from 8 healthy individuals have been included as control.

Informed consent has been taken from all patients and control.

All the biopsied warty lesions were subjected to routine light microscopic examination of hematoxylin and eosin-stained sections to confirm the diagnosis before immunohistochemical staining.

Immunohistochemistry:

i. Four-micron thick tissue sections cut from the representative paraffin-embedded tissue blocks, overlaid on APES (Sigma, St. Louis, USA) coated slides, were deparaffinized (2 changes of Xylene X 5 minutes each, 1 change of acetone X 1 min) followed by rehydration in decreasing ethanol concentrations (95% ethanol X 3 mins, 70% ethanol X 3 mins, distilled water X 1 min).

ii. For staining with all the antibodies, the tissue sections were subjected to antigen unmasking by heating the sections immersed in 10 mM citrate buffer pH 6.0 (2.1 gm. of anhydrous citric acid crystals dissolved in 1L of distilled water and pH adjusted to 6.0) inside a 600 watt microwave oven in full power for 35 minutes, allowed to cool to room temperature and then washed briefly with 0.05 M Tris-Hcl

buffer pH 7.4.

iii. Endogenous peroxidase activity was then quenched by immersing the sections in methanolic H2O2 (1 part 3% H2O2 plus 4 parts absolute methanol) for 30 minutes. After brief rinsing, the sections were placed in 0.05 M Tris-Hcl buffer pH 7.4 for 10 minutes.

iv. Sections were then overlaid with adequate amount of primary antibody diluted optimally using 0.05 M Tris-Hcl buffer pH 7.4 containing 1% bovine serum albumin (Sigma, St. Louis, USA) followed by incubation at 40C overnight.

v. The slides were then washed with three changes (5 mins each) of 0.05 M Tris-Hcl buffer pH 7.4 followed by incubation for 30 minutes at room temperature after application of biotinylated secondary (link) antibody in phosphate buffered saline containing carrier protein and 15 mM sodium azide (LSAB Plus Kit, DAKO, Denmark).

vi. After three washings (5 mins each) in Tris-Hcl buffer, peroxidase conjugated streptavidin was applied to cover the specimens and incubated at room temperature for 30 minutes.

vii. Slides were rinsed with 3 changes of Tris-Hcl buffer for 5 mins each. Sections were then covered with substrate chromogen solution prepared freshly by dissolving 1 mg of 3,3?-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, USA) in 1 ml of 0.05 M Tris-Hcl buffer pH 7.4 containing 1 ?l of hydrogen peroxide. The slides were incubated at room temperature for 5 to 10 minutes under microscopic control till the optimal development of brown colored peroxidase reaction product.

viii. After rinsing in distilled water, the sections were lightly counterstained with Harris' hematoxylin, followed by mounting with cover slips with DPX as mounting medium.

ix. Precaution was taken so that drying of tissue sections strictly did not occur at any time during the entire procedure of immunostaining. All incubations were done inside humid chambers.

x. Controls: During each batch of staining, positive and negative controls appropriate for the particular antibody were incorporated.

The details of primary and secondary antibodies used in the study are shown in **table (1)**.

Antigen	1ry antibody			2ry antibody		
	species	Conc.	manufacturer	species	dilution	manufacturer
survivin	Mouse	2-4 µg/ml	Abcam*	Rabbit	0.73611	Abcam
p-53	Mouse	5 µg/ml	Abcam	Rabbit	0.73611	Abcam
Bcl-2	Goat	15 µg/ml	R&D Systems**	Rabbit	0.73611	Abcam
PCNA	Mouse	1.15278	Abcam	Rabbit	0.73611	Abcam

Table (1): The details of antibodies utilized in the study.

The slides were examined for both routine histopathology and immunohistochemistry using Olympus light microscope equipped with SIS Image Analysis Computer System for the purpose of quantitative assessment of immunohistochemical reactivity. Examination was done under X40, X100, X200 and X400 magnification.

Scoring method:

The scoring method for different markers was modified from that described by Sinicrope et al.. (1995).[38]Four sections per specimen have been examined for each marker evaluated in the study. The mean percentage of positive tumor cells was determined in at least five fields per section (at 400-fold magnification) and assigned to one of the following 8 categories: 0, <5%; 1, 5-12.5%; 2, 12.5-25%; 3, 25-37.5%; 4, 37.5-50%, 5, 50-67.5%, 6, 67.5-75%, 7, 75-87.5%, 8, >87.5%. (N.B. the score refers to the percent of positively stained cells). The intensity of immunostaining was scored as follows: 1+, weak; 2+, moderate; and 3+, intense. Because the lesions showed heterogeneous staining, the dominant pattern was used for scoring. The scores indicating percentage of positive cells and staining intensity were multiplied to produce a weighted score for each field. The average weighted score for the 20 (X200) fields examined for each specimen (5 fields/section X 4 sections) is then calculated. Cases with weighted scores <1 were defined as negative, and cases were otherwise defined as positive.

Statistical analysis.

Comparative analysis between two groups was done through unpaired T-test and chi-square test using graphpad software downloaded from the website: http://www.graphpad.com[39]

Correlation between different parameters was done through graphpad software downloaded from the website: http://calculators.stat.ucla.edu/correlation.php[40]

Results

The study included 15 patients and 8 control subjects of matched age and sex. Eleven clinically typical lesions of verruca vulgaris (from 8 patients) and 11 typical lesions of condyloma acuminata (from 7 patients) have been biopsied in addition to 8 biopsies from matched areas of normal skin (from 8 healthy subjects of matched age and sex). The clinical data of the patients and control group are shown in **table (2)**.

	Control (n=8)		Verruca vulgaris (n=8)		Condyloma Acuminata (n=7)	
Age (years)	Range	Mean +/-SD	Range	Mean +/-SD	Range	Mean +/-SD
	12 - 45	23.7 +/- 15.3	11 - 48	20.4 +/- 17.8	21 - 42	30.2 +/- 9.8
Sex	Males 4	Females 4	Males 5	Females 3	Males 5	Females 2
Number of biopsies	8		11		11	
Site of biopsied lesions	Hands (2) Thigh (3) Pubic area (2) Scrotum (1)		Hands (4) Fingers (2) Legs (2) Abdomen (2) Pubic area (1)		Pubic area (4) Genitalia (5) Thigh (2)	
Approximate duration of lesions (months)	range	Mean +/-SD	range	Mean +/-SD	range	Mean +/-SD
	------	-------	1 - 20	8.3 +/- 7.4	2 - 24	6.1 +/- 10.7

Table (2): clinical data of the study material.

Immunohistochemical examination of both V.V. and C.A. lesions could reveal significantly increased reactivity for survivin as well as for p-53 compared to normal skin (in terms of percent positive lesions and of average weighted score). Normal skin was almost negative for both markers in most biopsies studied. Lesions of C.A. were more reactive for both markers than those of V.V. but the difference was statistically significant only for p53. ($p<0.05$)

The reactivity for survivin was diffuse all over the epidermis but more prominent in the lower epidermal layers. The cellular staining pattern was finely granular and more nuclear than cytoplasmic.

For p-53, the staining was also diffuse all over the epidermis (basal and suprabasal). At the cellular level, staining was either granular or homogeneous and compact and was either nuclear or both nuclear and cytoplasmic (especially in areas of heavy staining).

Anatomic correlation could be observed in many lesions between areas of intense immunoreactivity for both markers.

For Bcl-2, staining in both types of verrucous lesions was weak and did not significantly differ from corresponding staining of normal skin. The staining in all skin specimens examined was mainly in the basal or immediate suprabasal layers and always cytoplasmic (perinuclear).

Both V.V and C.A. lesions showed significantly enhanced staining for PCNA in comparison to normal skin, with V.V. lesions more reactive to PCNA than lesions of C.A. although the difference was not statistically significant between V.V. and C.A. Reactivity was nuclear, homogeneous and most marked in the basal layer and gradually becomes less higher up in the epidermis.

See **figs. (1 to 3)**.

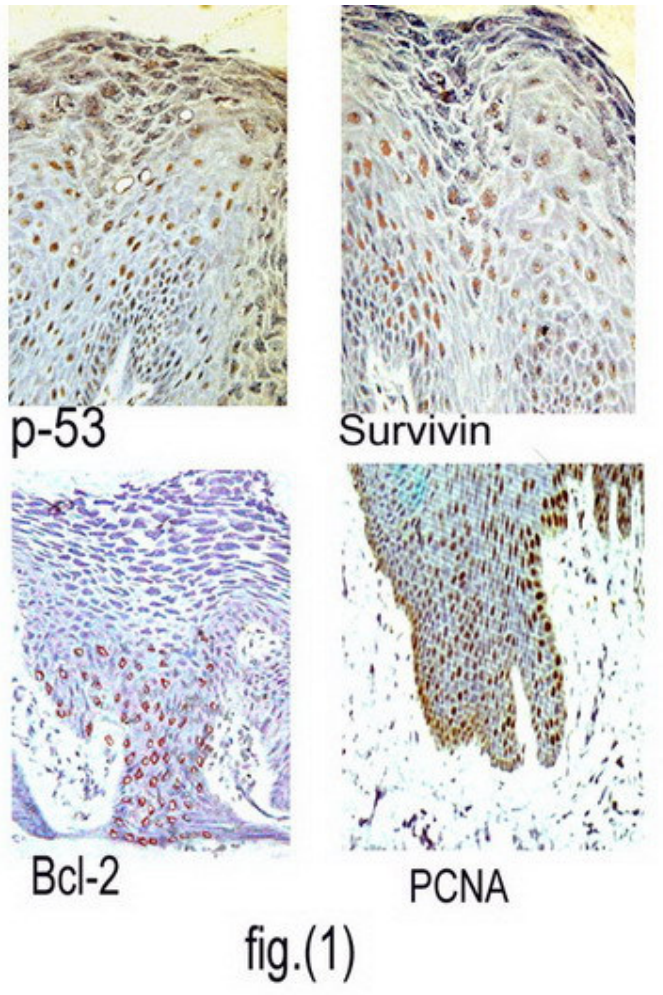

Fig.(1): Immunohistochemical staining of verruca vulgaris (V.V.) lesions showing markedly enhanced expression of P-53 (throughout the epidermis, both nuclear and cytoplasmic), of survivin (throughout the epidermis, mainly nuclear and to a less extent cytoplasmic) and of PCNA (mainly in lower epidermis & nuclear). No significant staining for Bcl-2 could be observed.

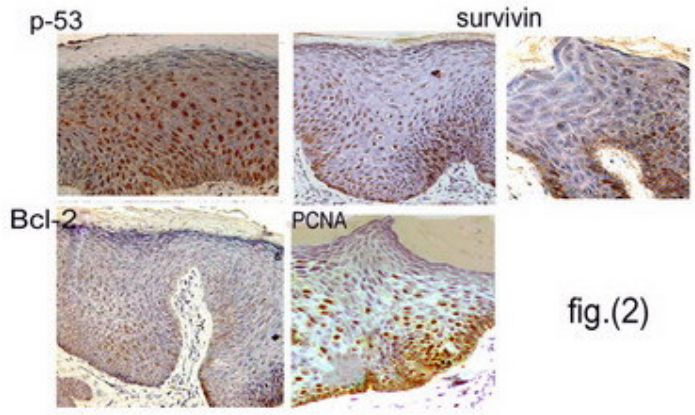

Fig 2: Immunohistochemical staining of condyloma acuminata (C.A.) lesions showing markedly enhanced expression of P-53 (throughout the epidermis, both nuclear and cytoplasmic), of survivin (throughout the epidermis, mainly nuclear and less prominently cytoplasmic) and of PCNA (mainly in lower epidermis & nuclear). No significant staining for Bcl-2 could be observed.

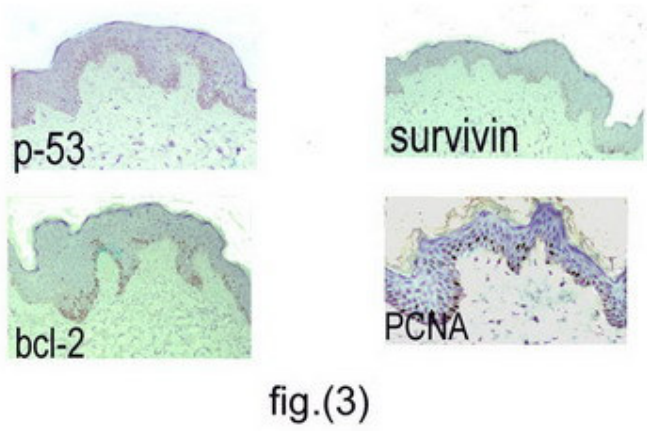

Fig.(3): Immunohistochemical staining of normal skin showing no staining for p-53 or survivin. Poor staining could be seen for Bcl-2 (in lower epidermis - mainly perinuclear). Staining for PCNA could be seen clearly in lower epidermis

Immunoreactivity for survivin showed strongly positive correlation with that of p-53 in both types of verrucous lesions. No correlation could be observed between survivin and other markers evaluated in the study. **Table (3)c**

Results of immunohistochemical examination are shown in **tables (3)a, b & c**

	Normal skin	Verruca vulgaris		Condyloma acuminata	
weighted score	Mean +/- SD	Mean +/- SD	***p-value***	Mean +/- SD	***p-value***
survivin	0.0 +/- 0.0	3.2 +/- 3.8	**<0.05***	7.6 +/- 7.5	**<0.05***
p-53	0.0 +/- 0.0	2.8 +/- 2.9	**<0.05***	7.3 +/- 6.1	**<0.01***
Bcl-2	1.0 +/- 1.1	1.7 +/- 3.4	**>0.05**	0.9 +/- 1.1	**>0.05**
PCNA	2.1 +/- 1.1	11.73 +/- 7.0	**<0.01***	8.7 +/- 6.2	**<0.01***

* = statistically significant

Table (3)a Results of immunohistochemical examination (Weighted score).

	Normal skin	Verruca vulgaris		Condyloma acuminata	
% of positive lesions	percent	percent	***p-value***	percent	***p-value***
survivin	(0/8) 0.0%	(7/11) 63.6%	**<0.05***	(8/11) 72.7%	**<0.01***
p-53	(0/8) 0.0%	(7/11) 63.6%	**<0.05***	(10/11) 90.9%	**<0.001***
Bcl-2	(5/8) 62.5%	(3/11) 27.3%	**>0.05**	(6/11) 54.5%	**>0.05**
PCNA	(8/8) 100%	(11/11) 100%	**>0.05**	(11/11) 100%	**>0.05**

* = statistically significant

Table (3)b Results of immunohistochemical examination (Number & percentage of positive lesions)

	survivin	p-53	Bcl-2	PCNA
survivin		0.97*	0.21	0.05
p-53			-0.17	0.003
Bcl-2				0.39
PCNA				

* = statistically significant
(Figures shown refer to correlation coefficient)

Table (3)c Results of immunohistochemical examination (Correlation matrix)

Fig.(4) illustrates comparative staining scores for different markers in V.V., C.A. and control.

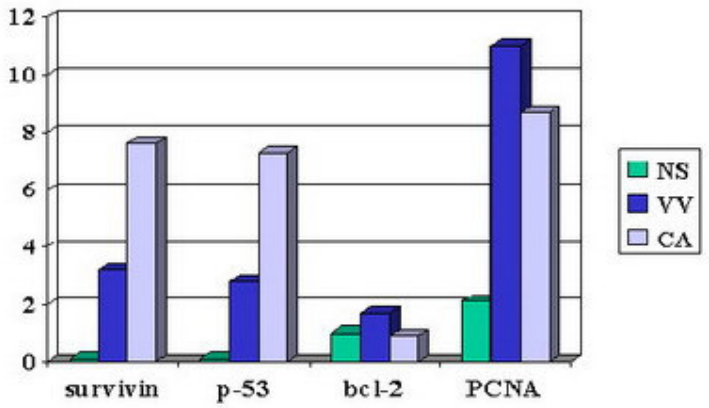

Fig.(4): comparative expression of survivin, p53, Bcl-2 and PCNA in VV, CA and control skin

Discussion

Among the regulatory proteins evaluated in the study, only overexpression of survivin and PCNA seems to be of relevance to the hyperplastic nature of HPV lesions. On the contrary, overexpression of p-53 (with its known pro-apoptotic and anti-proliferative activities)[23]and weak expression of Bcl-2 (with its known anti-apoptotic properties),[34]may represent unexpected findings in a hyperplastic lesion and undermine the possibility of a major role of these proteins in the evolution of HPV-related lesions.

Similar findings in both types of HPV-related lesions may suggest similar evolutionary pathway of these lesions in spite of different serotype of the causative virus.4 It may also cast doubts on the prognostic value of some of these markers in distinguishing between benign and potentially malignant lesions. Although survivin overexpression has been usually linked to malignancy, occurrence in totally benign neoplasms could be shown in this study as well as in some previous studies both in cutaneous and non-cutaneous lesions.[8]This would compromise its prognostic value suggested in earlier studies as an indicator of high grade malignancy[41],[42],[43],[44],[45],[46],[47] and argues that inhibition of apoptosis and/or alteration of cell cycle by survivin is not necessarily equivalent to (or followed by) cell transformation. The possibility, however, is there of low risk serotypes as the causative agent of CA in our patients with no oncogenic potential. Moreover, the possibility of the presence of mutant forms of some of these markers (especially for p-53) that are non-functional but overexpressed can not be ruled out as was confirmed in some other neoplasms.[25], [26]

In the present study, there was poor correlation between the proliferation index (as indicated by PCNA score) and that of survivin indicating that possible contribution of survivin to hyperplastic skin reaction in HPV-induced neoplasms may be mediated through inhibiting apoptosis rather than by inducing cell division. In fact, some HPV-related benign lesions have been shown in other studies[21]to exhibit low apoptotic index comparable to that of normal skin. Survivin may be one of the factors contributing to this suppression of apoptosis in spite of the viral insult to keratinocytes. On the other hand, hyperproliferation that has been confirmed in this study (as shown by PCNA overexpression) may be mediated through other mechanisms.

Although the overall average score of survivin positive cells is significantly high, some lesions were totally negative for survivin. This can be explained in several ways. First of all is the age (or stage) of lesion as tissue hyperplasia may be a consequence of transient state of imbalance between tissue proliferation and apoptosis after which maintenance of the stationary state may just necessitate reestablishment of the original balance between these two processes. This may be expected in lesions like viral warts where -in most cases- the lesion exhibits an initial state of expansion followed by cessation of growth in contrast to the relentlessly expanding malignant neoplasms.[48]Another possible reason is the probable difference in the causative viral serotype as clinically similar HPV lesions can be caused by different serotypes that have different gene characteristics and more importantly different potential (or mechanisms) of inducing cell transformation and/or altering host cell dynamics.[49]

Nevertheless, the demonstration of increased reactivity to PCNA

(as regards the average weighted score of staining in all studied verrucous lesions) suggests a continuous state of hyperproliferation that is probably necessary to maintain the hyperplastic state in the face of other opposing mechanisms (e.g. overexpressed p-53).

Again, lack of significant Bcl-2 expression would be inconsistent with a major role of Bcl-2 anti-apoptotic action in the evolution of HPV-related lesions. The reduced levels of Bcl-2 may be related in some way or another to the upgraded expression of p-53 (a known inhibitor of Bcl-2 expression)[50], although no clear correlation (negative or positive) could be demonstrated in this study between these two markers.

The minimal expression of Bcl-2 agrees with previous studies carried out on benign HPV lesions,[51]reporting reduced Bcl-2 in contrast to normal BAX expression in the lesional skin. This does not exclude the presence of another HPV-induced anti-apoptotic mechanism independent of (or bypassing) that mediated by Bcl-2 (e.g. at the caspase level as suggested for survivin).[8]

Another study carried out on a variety of cutaneous neoplasms could show similar reduction of Bcl-2 within V.V. and C.A. lesions while the malignant (or premalignant) nature of other tumors subjected to evaluation (not the mere hyperplasia) was associated with increased Bcl-2 expression.[52]

The strikingly enhanced expression of P-53 in HPV lesions shown in the present study contradicts with previous reports of reduced p-53 immunoreactivity in HPV-induced lesions.[52]Likely explanation to the observed expression is the possible lack of malignant potential of the biopsied lesion (probably related to the underlying serotype of the virus which has not been assessed in the current study and also in most of the previous related studies). Epidemiological difference in the prevalence of different human papilloma viral serotypes in different communities (with different potential of malignant transformation) may be one possible cause. The loss of p-53 has been typically reported in (and also etiologically linked to) those lesions with potential of malignant transformation. In cases of HPV-induced lesions, high risk serotypes were reported to induce cell transformation through expression of 2 main gene products namely E-6 and E-7genes. E-6 gene products -in particular- have potent inhibitory effect on p-53 expression and p-53 action (through inhibiting p-21).[49]So, the demonstration of p-53 in most biopsied lesions in the present study could point to benign nature of the underlying viral serotype.

Another controversy is the contrast between overexpression of p-53 (a potent anti-proliferative and pro-apoptotic factor)[23]and the

nature of warts as hyperplastic lesions. The answer may again reside in the age (or stage) of the lesion where stimulation of proliferation and/or inhibition of apoptosis seem to be necessary only in the initial stage of evolution after which a stationary state is reached where maintenance of the lesion may just require re-institution of the balance between proliferation and cell deletion by apoptosis. In harmony with this possibility is the well-established link between apoptosis and proliferation where enhanced proliferation has been shown to stimulate enhanced apoptosis under physiological and also in many pathological conditions.[53]

Some studies could demonstrate the ability of p-53 to suppress HPV-related hyperproliferation and made the suggestion that at a certain stage of viral cycle, a feedback loop begins to operate in which some viral proteins (e.g. E1 or E2) may act to block the initial viral suppressor effect on p-53 action &/or expression giving it a chance to check the hyperactive cell proliferative process.[54]

However, It is possible that the contribution of p-53 to the process of evolution (or maintenance) of wart lesions is a minor one or is overcome by opposing and more effective mechanisms. The presence of p-53 in a mutant form that is overexpressed but non-functional is another possibility that, however, could not be verified by our conventional immunohistochemical technique. If true, the question remains as whether the mutation is intrinsic or induced by the viral infection itself.

Another striking finding in the present study is the strongly positive correlation between p-53 and survivin. This is also unexpected keeping in mind the inhibitory effect which p-53 is supposed to exert on survivin expression.[8]

Again, the existence of p-53 in a mutant ineffective form is one possibility.[25]Another explanation is the presence of many other (& probably more potent) regulators of survivin expression apart from p-53 including other oncogene products and a number of growth factors. The relative importance of these different factors and their possible alteration in HPV-induced lesions are still to be elucidated but seem to vary even from one type of lesions to another.[8]

In addition to the negative effect p53 has on survivin expression,[8]both factors have antagonistic effect on cell dynamics. P-53 appears to be capable of inducing cell cycle arrest and/or apoptosis through a large number of regulatory pathways. Some of these can intersect with the action of survivin (e.g. as regards caspase inhibition).[55]Although the exact mechanism(s) [or site(s)] of action of survivin are not yet settled,[8]some of these mechanisms may be probably capable of either bypassing or overcoming the opposing action of p53 at least in case of HPV-induced lesions.

As p53 was overexpressed in both types of HPV-lesions evaluated in the study, it may not be a sinister indicator of cell transformation. It would rather represent a natural cytoprotective reaction to the stress imposed on the cell as a result of viral insult. The overexpressed p53 may then start (or try to start) a state of cell cycle arrest so as to give a chance for DNA repair &/or cell apoptosis.[23]Given the persistent nature of the cellular insult (in the form of persistent viral infection), the overexpression may be also persistent but may be somewhat ineffective in halting the hyperplastic tissue reaction.

Conclusions and Recommendations

Although the study could strongly suggest involvement of some proliferative and apoptotic mediators (in particular survivin) in the pathogenesis of tissue reaction of HPV-induced lesions, the exact sequence of events seems to be more complicated and may involve a large number of factors interacting in a sophisticated way. In-vitro studies may be more helpful in studying the role of each factor in isolation. There may be also a great need for long term studies to correlate the immunohistochemical findings of the early stages with the long term clinical course of the lesions and in particular in relation to the potential for malignant transformation and also with the susceptibility to spontaneous (or immune-mediated) regression.

Furthermore, correlation with the underlying serotype can not be overemphasized as the clinically similar group of lesions could be largely heterogeneous when considering the underlying viral serotypes. Establishment of a clear view of the specificity or sensitivity of any of the studied factors as a prognostic marker of malignancy may necessitate long term as well as large scale studies on a wide variety of lesions before firm conclusions can be made.

Lastly, therapeutic manipulation of some of these pro- or anti-apoptotic factors stands as an attractive option in the management of this type of skin lesions.

References

1. Reed, JC. (2000): Mechanisms of apoptosis. Am. J. Pathol. (157):1415.

2. Reed, J.C. (1999): Dysregulation of apoptosis in cancer. J. Clin. Oncol. (17): 2941

3. Cory, S., Huang, D.C., Adams, J.M. (2003): The Bcl-2 family: roles in cell survival and oncogenesis. Oncogene; (22): 8590

4. Beutner, K.R. and Tyring, S, (1997): Human papillomavirus and

human disease. Am. J. Med. (102)5A: 9-15

5. Butel, J. (2000): Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. Carcinogenesis (21): 405-426.

6. Van Doornum, G.J. Prins, M. Juffermans, L.H., Hooykaas, C., van den Hoek, J.A., Coutinho, R.A. and Quint, W.G. (1994): Regional distribution and incidence of human papillomavirus infections among heterosexual men and women with multiple sexual partners: a prospective study Genitourinary Medicine, (70)4: 240-246

7. Arends, M.J., Wyllie, A.H., Bird, C.C. (1990): Papillomaviruses and human cancer. Hum. Pathol. (21)7: 686-98

8. Johnson, M. E. and Howerth, E. W. (2004): Survivin: A Bifunctional Inhibitor of Apoptosis Protein Vet. Pathol. (41): 599-607

9. Reed, J.C. and Bischoff, J.R. (2000): BIRinging chromosomes through cell division-and survivin' the experience. Cell (102): 545-548

10. Li, F. and Altieri, D.C. (1999): The cancer antiapoptosis mouse survivin gene: characterization of locus and transcriptional requirements of basal and cell cycle-dependent expression. Cancer Res. (59): 3143-3151

11. Zwicker, J., Lucibello, F.C. and Wolfraim, L.A. (1995): Cell cycle regulation of the cyclin A, cdc25C, and cdc2 genes is based on a common mechanism of transcriptional repression. EMBO. J. (14): 4514-4522

12. Ambrosini, G., Adida, C. and Altieri, D.C. (1997): A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat. Med. (3): 917-921

13. Riedl, S.J., Renatus, M., Schwarzenbacher, R., Zhou, Q., Sun, C., Fesik, SW., Liddington, R.C., and Salvesen, G.S. (2001): Structural basis for the inhibition of caspase-3 by XIAP. Cell (104): 791-800

14. O'Connor, D.S., Grossman, D., Plescia, J., Li, F., Zhang, H., Sci Villa, A., Tognin, S., Marchisio, P.C. and Altieri, D.C. (2000): Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc. Natl. Acad. (97): 13103-13107

15. Marusawa, H., Matsuzawa, S., Welsh, K., Zou, H., Armstrong, R., Tamm, I. and Reed, J.C. (2003): HBXIP functions as a cofactor of survivin in apoptosis suppression. EMBO. J. (22): 2729-2740

16. Song, Z., Yao, X. and Wu, M. (2003): Direct interaction between

survivin and Smac/DIABLO is essential for the anti-apoptotic activity of survivin during taxol-induced apoptosis. J. Biol. Chem. (25): 23130-23140

17. Carter, B.Z., Milella, M., Altieri, D.C.and Andreeff, M. (2001): Cytokine regulated expression of survivin in myeloid leukemia. Blood (97): 2784-2790

18. Gianani, R., Jarboe, E., Orlicky, D., Frost, M., Bobak, J., Lehner, R. and Shroyer, K.R. (2001): Expression of survivin in normal, hyperplastic, and neoplastic colonic mucosa. Hum. Pathol. (32): 119-125

19. Chiou, S., Moon, W.S., Jones, M.K. and Tarnawski, A.S. (2003): Survivin expression in the stomach: implications for mucosal integrity and protection. Biochem. Biophys. Res. Commun. (305): 374-379

20. Frost, M., Jarboe, E.A., Orlicky, D., Gianani, R., Thompson, L.C., Enomoto, T. and Shroyer, K.R. (2002): Immunohistochemical localization of survivin in benign cervical mucosa, cervical dysplasia, and invasive squamous cell carcinoma. Am. J. Clin. Pathol. (117)5: 738-744,

21. Bowen, A.R., Hanks,A.N., Murphy, K.J., Florell, S.R. and Grossman, D. (2004): Proliferation, Apoptosis, and Survivin Expression in Keratinocytic Neoplasms and Hyperplasias. Am. J. Dermatopathol. (26): 177-181

22. Grossman, D., McNiff, J.M. and Li, F. (1999). Expression and targeting of the apoptosis inhibitor, survivin, in human melanoma. J. Invest. Dermatol. (113): 1076-1081

23. Janus, F., Albrechtsen, N., Dornreiter, I., Wiesmuller, L., Grosse, F.and Deppert, W.(1999): Cell. Mol. Life. Sci. (55): 12-27

24. Lyons, . S.K. and Clark, A.R. (1997): Apoptosis and Carcinogenesis. British medical Bulletin (52)3: 554-569

25. Soussi, T. (2000): The p53 tumor suppressor gene: from molecular biology to clinical investigation. Ann. NY. Acad. Sci. (910): 121-39

26. Bargonetti, J. and Manfredi, J.J. (2002): Multiple roles of the tumor suppressor p53. Curr. Opin. Oncol. (14): 86-91

27. Vousden, K.H. (2002): Activation of the p53 tumor suppressor protein. Biochim. Biophys. Acta. (1602): 47-59

28. Alarcon-Vargas, D. and Ronai, Z. (2002): p53-Mdm2--the affair

that never ends. Carcinogenesis (23):541-7.

29. Momand, J., Wu, H.-H. and Dasgupta, G. (2000):. MDM2--master regulator of the p53 tumor suppressor protein. Gene (242)1: 15-29

30. Kaelin, W.G. (1999): The emerging p53 gene family. J Natl. Cancer Inst. (91): 594-8

31. Gong, B. and Almasan, A. (1999): Differential upregulation of p53-responsive genes by genotoxic stress in hematopoietic cells containing wild-type and mutant p53. Gene Expr. (8): 197-206

32. Kelman, Z. (1997): PCNA: structure, functions and interactions. Oncogene (14): 629-40

33. Hockenberry, D., Zutter, M., Hickey, W., Nahm, M. and Korsmeyer, S. (1991): BCL2 protein is topographically restricted in tissues characterized by apoptotic cell death. Proc. Natl. Acad. Sci. (88): 6961-965

34. Cotran, R.S., Kumar, V., Collins, T. and Robbins, S.L. (1999): In Pathologic Basis of Disease. R. S. Cotran, V. Kumar, T. Collins. And S.L. Robbins -6th edition W.B. Saunders and Co., Pp: 18-25.

35. Haake, R.A. and Polakowska, R.R. (1993): Cell death by apoptosis in epidermal biology. J. Invest. Dermatol. (101): 107-112

36. Gandarillas, A (2000):. Epidermal Differentiation, Apoptosis, and Senescence: Common Pathways? Experimental Gerontology (35)1: 53-62

37. Arends, M.J., Wyllie, A.H. and Bird, C.C. (1990): Papillomaviruses and human cancer. Hum. Pathol. (21)7: 686-98

38. Sinicrope, F.A., Ruan, S.B., Cleary, K.R., Stephens, L.C., Lee, J.J. and Levin, B. (1995): bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer. Res. (55): 237-241

39. http://www.graphpad.com/quickcalcs/ttest1.cfm39

40. http://calculators.stat.ucla.edu/correlation.php40

41. Saitoh, Y. and Yaginuma, Y. and Ishikawa, M. (1999): Analysis of Bcl-2, Bax and survivin genes in uterine cancer. Int. J. Oncol. (15):137-141

42. Sarela, A.I., Scott, N., Ramsdale, J., Markham, A.F.and Guillou, P.J. (2001): Immunohistochemical detection of the anti-apoptosis protein, survivin, predicts survival after curative resection of stage II

colorectal carcinomas. Ann. Surg. Oncol. (8): 305-310

43. Satoh, K., Kaneko, K., Hirota, M., Masamune, A., Satoh, A. and Shimosegawa, T. (2001): Expression of survivin is correlated with cancer cell apoptosis and is involved in the development of human pancreatic duct cell tumors. Cancer (92): 271-278

44. Schmidt, S.M., Schag, K., Muller, M.R., Weck, M.M., Appel, S., Kanz, L., Grunebach, F. and Brossart, P. (2003): Survivin is a shared tumor-associated antigen expressed in a broad variety of malignancies and recognized by specific cytotoxic T cells. Blood (2):571-576

45. Shi, Y. (2000): Survivin structure: crystal unclear. Nat Struct. Biol. (7): 620-623

46. Skoufias, D.A., Mollinari, C., Lacroix, F.B. and Margolis, R.L. (2000): Human survivin is a kinetochore-associated passenger protein. J. Cell Biol. (151): 1575-1582,

47. Smith, S.D., Wheeler, M.A., Plescia, J., Colberg, J.W., Weiss, R.M. and Altieri, D.C. (2001): Urine detection of survivin and diagnosis of bladder cancer. JAMA (285): 324-328,

48. Campion, M.J. (1987): Clinical manifestations and natural history of genital human papillomavirus infection. Obstet Gynecol Clin North Am ;(14)2: 363-88.

49. Kessis, T.D., Connolly, D.C., Hedrick, L. and Cho, K.R. (1996): Expression of HPV16 E6 or E7 increases integration of foreign DNA. : Oncogene. (13)2: 427-31

50. Wu, Y., Mehew, J.W., Heckman, C.A., Arcinas, M. and Boxer, LM. 2001 Negative regulation of bcl-2 expression by p53 in hematopoietic cells Oncogene. (20)2: 240-51.

51. Erkek, E., Bozdogan, O., Atasoy, P., Birol, A. and Kocak, M. (2004): BCL-2-related apoptosis markers in cutaneous human papillomavirus-associated lesions; Am. J. Dermatopathol. (26)2: 113-8

52. Hussein, M. R., Al- Badaiwy, Z. H. and Guirguis, M. N. (2004): Analysis of p53 and bcl-2 protein expression in the non-tumorigenic, pretumorigenic, and tumorigenic keratinocytic hyperproliferative lesions. Journal of Cutaneous Pathology (31)10: 643

53. Guo, M. and Hay, B.A. (1999): Cell proliferation and apoptosis. Curr. Opin. Cell. Biol. (11): 745-52.

54. Lepik, D., Ilves, I., Kristjuhan, A., Maimets, T. and Ustav, M.

(1998): p53 Protein Is a Suppressor of Papillomavirus DNA Amplificational Replication. J Virol. (72)8: 6822-6831

55. Oren, M. and Rotter, V. (1999): Introduction: p53- the first twenty years. Cell. Mol. Life. Sci. (55): 9-11

Abbreviations:

APAF-1: Apoptosis protease activating factor
ATM: Ataxia-telangiectasia mutant protein
ATR: Ataxia telangiectasia and Rad3 related protein
BAX: Bcl-2 associated x protein
Bcl-2: B- cell leukemia/ lymphoma-2
C.A: Condyloma acuminata
GADD45: Growth Arrest and DNA Damage--inducible Gene 45
HPV: Human papilloma virus
IAP: inhibitors of apoptosis proteins
MDM2: Mouse double minute 2
NS: Not significant
PCNA: proliferating cell nuclear antigen
STAT-3: signal transducer and activator of transcription 3
V.V.: Verruca vulgaris

الملخص العربي

دراسة لمادة السيرفايفين فى حالات الثآليل الشائعة والثآليل التناسلية :

درجة التناسب مع دالات التكاثر والاستماتة (الموات الخلوى المبرمج) الأخرى

د/أحمد إبراهيم رشيد د/زينب عبد الرحمن قمر*

قسم الأمراض الجلدية والتناسلية – جامعة عين شمس

* قسم الباثولوجى – جامعة عين شمس

فى هذه الدراسة تم فحص 11 اصابة ثآليل تناسلية (لقموم مؤنف) و 11 اصابة ثآليل شائعة و 8 عينات من جلد سليم و ذلك بتقنية الفحص الكيميائى النسيجى المناعى لتقدير درجة تواجد مادة السيرفايفين فى الجلد بالاضافة الى بعض دالات التكاثر والموت الاضمحلالى (الموات الخلوى المبرمج) الأخرى وتشمل p-53 و Bcl-2 و PCNA

ولقد اظهرت الدراسة زيادة واضحة فى مستوى التصبغ المناعى لمادتى السيرفايفين و p-53 فى نوعى الثآليل بالمقارنة بالجلد الطبيعى. كما أ ظهرت الدراسة زيادة فى مستويات PCNA فى كل من نوعى الثآليل (الشائعة و التناسلية) وكانت الزيادة أكبر فى النوع الأول بينما لم تظهر الدراسة مستويات ذّات مغزى لمادة Bcl-2 سواء فى الثآليل الشائعة أو التناسلية

وبناء على هذه النتائج يمكن افتراض دور لمادة السيرفايفين فى نشوء هذه الاصابات التناسلية بينما يمكن تصور دور لمادة p-53 فى اعادة التوازن لمعدل التكاثر النسيجى لخلايا البشرة بعد اصابتها بالفيروس.