The Role Of IMP3 And BCL2 In Differentiating Between Irritated Seborrheic Keratosis, Insitu And Invasive Squamous Cell Carcinomas Of The Skin

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Abstract

Introduction: The histological distinction between squamous cell carcinoma both in-situ and invasive from their benign mimic; irritated seborrheic keratosis is still a difficulty. The aim of this study is to investigate the diagnostic utility of Insulin-like growth factor II mRNA-binding protein 3 (IMP3), and B-cell lymphoma 2 (BCL2) in differentiating aforementioned cutaneous tumors**. Materials and Methods:** This is a retrospective study to include 50 cases, 25(50%) cases of invasive squamous cell carcinoma (SCC), 10 (20%) cases of squamous cell carcinoma in-situ (SCCIS), and 15 (30%) cases of irritated seborrheic keratosis (ISK). The immunohistochemical expression of IMP3 and BCL2 was studied **(9) Results:** BCL2 was expressed in 12/15(80%) ISK cases compared to 3/10 (30%) in SCCIS and 4/25 (16%) in invasive SCC cases ($p < 0.01$). IMP3 was expressed (score, 3+) in 20/25(80%) of SCC , 6/10 (60%) of SCCIS,.while,11/15 (73.3%) ISK cases were negative for IMP3 ($p < 0.01$).The combined expression of both markers in differentiating SCC (invasive and in-situ) from ISK was statistically significant ($p<0.001$). **Conclusion:** The immunohistochemical detection of IMP3and/or BCL2expression might be of diagnostic value in differentiating ISK from both invasive SCC and SCCIS but has no significant role in distinguishing invasive SCC from SCCIS.

Keywords: irritated seborrheic keratosis, squamous cell carcinoma, IMP3, BCL2

Introduction:

Squamous cell carcinoma (SCC) is one of the most common malignant skin tumors after basal cell carcinoma and malignant melanoma [**1**]. It is locally invasive, and

most cases are readily diagnosable by microscopic examination [**2**]. Squamous cell carcinoma in situ (SCCIS) represents a superficial variant of skin cancer that can become invasive in a small subset of cases [**3**].

Seborrheic keratosis (SK) ranks as the most common cutaneous neoplasm and is a benign tumor with hyperplasia of the epidermis. The development of SK is a clonal process involving the proliferation of basal keratinocytes with the formation of pseudohorn cysts [**4**].There are many variable histologic patterns of SK, including acanthotic hyperkeratotic, clonal, reticulated, pigmented, and irritated types[**5**]. Irritated seborrheic keratosis (ISK) shows squamous eddies formed by aggregates of eosinophilic squamous cells in whorls, and the dermis is infiltrated by inflammatory cells in a lichenoid pattern [**6**]

The microscopic diagnosis of SCC is challenging due to benign mimics, especially when the specimen is minute or only a small portion of a superficial surgical biopsy is received [7].These benign mimics include ISK, where the thickened epidermis and whorled accumulations of keratinocytes can be interpreted as SCCIS [**4**] or the squamous eddies may be confused with the horn pearls of SCC [**7,8**]

Since ISK shares clinical and histopathological similarities with SCCIS and SCC (Table (1), Fig.1 A–C), the distinction between them can occasionally be conflicting, and a correct diagnosis must be made because the treatment strategies differ. Thus, the use of immunohistochemical markers has been advised, particularly when diagnosing lesions with conflicting characteristics [**9**]

B cell lymphoma (BCL2) is a major anti apoptotic protein that is responsible for maintaining stem cells to sustain self-renewal [**10**] ,It is positively expressed in benign skin lesions [**11**]

Insulin-like growth factor II mRNA-binding protein 3 (IMP3), also known also (KOC), is an oncofetal protein associated with cell proliferation [**12, 13**]

The overexpression of IMP3 has been utilized to differentiate between benign and malignant tumors in many types of cancer, including SCC [**14**]

This immunohistochemical investigation studied BCL2 and IMP3 expression in SCC, SCCIS, and ISK to determine whether they

could discriminate SCC (both invasive and in-situ) from ISK.

Materials and Methods

Study approval and design

This retrospective histopathological study was carried out in pathology department – Benha faculty of medicine approved by the Pathology Department and the Ethical Committee of Benha University Hospital, Egypt. We evaluated 50 cases of different skin lesions that occurred between January 2013 and December 2018, including, 25(50%) cases of SCC, 10 (20%) cases of SCCIS, and 15 (30%) cases of ISK. Of them, 35were excisional and 15 were punch biopsies. All specimens were fixed in formalin and embedded in paraffin wax blocks. We cut at 4–5µ thick sections and stained them with routine **hematoxylin & eosin** stain to revise the microscopic diagnosis.

Immunohistochemistry (IHC)

We employed a streptavidin-biotin technique (Lab Vision/NeoMarkers, CA, USA) for immunohistochemical analysis, according to the manufacturer's instructions (23) .

Then, we applied a 0.02% diaminobenzidine solution, followed by counterstaining using hematoxylin. Finally, the sections were dehydrated and mounted .For the negative control, the primary antibody was not added. Table (2)

Assessment of IMP3 and BCL2 immunoreactivity

IMP3 showed positive cytoplasmic expression and was graded as: negative (no positive expression), 1+ (1 -25% positive), 2+ (26% -50% positive), or 3+ (>50% positive) (**15)**

BCL2 was considered positive if both dendritic cells and keratinocytes showed cytoplasmic and or membranous staining and was scored as negative (<10% positive expression, or positive (>10% positive expression (**16)**

Statistical analysis

We used SPSS v20 (Statistical Package for Social Sciences; IBM Corp., NY, USA for all statistical analyses. The IHC data were analyzed using Fischer's exact test, and p-values ≤ 0.05 were considered statistically significant. Receiver operating characteristic (ROC) curve analysis was also performed.

Results

Patient characteristics

The mean age of the 50 cases under study was 47.12± 12.01 years (range, 24–62 years).

Immunohistochemical expression of BCL2 and IMP3

BCL2 was positively expressed in 12/15(80%) ISK cases (Fig.1D) compared with 3/10 (30%) and 4/25 (16%) SCCIS (Fig.1E) and invasive SCC (Fig–1F) cases, respectively, showing a statistically significant positive correlation ($p < 0.01$) (Table (3)

IMP3 was positively expressed (score,+3)in, 20/25 (80%) and 6/10 (60%) of SCC (Fig1G) and SCCIS (Fig,1-H) cases, respectively. IN contrast, most ISK cases (11/15, 73.3%) were negative (Fig. 1-I), showing a statistically significant direct correlation ($p<0.01$) (Table 3).

Combined expression of IMP3 and BCL2

An expression pattern of, IMP3 (+)/BCL-2 (−) was highly apparent in the SCCIS and SCC cases, while an expression pattern of IMP3(−)/BCL2(+) was particularly prominent in the ISK cases, with a statistically significant correlation ($p<0.001$). (Table 4)

ROC curve analysis

The ROC curve analysis illustrated the acceptable diagnostic performance of both IMP3 and BCL2 in discriminating patients with SCC and SCCIS from those with ISK.

For IMP3, the area under the ROC curve (AUC) for differentiating SCC and SCCIS from ISK was 0.924 and 0.823, respectively ($p < 0.001$).Regarding specificity and sensitivity,IMP3 was more specific and sensitive in discriminating SCC and SCCIS from ISK(specificity,20%,and sensitivity,96.0% and 80.0%, respectively).

For BCL2, the AUC for differentiating SCC and SCCIS from ISK was 0.18 and 0.25, respectively ($p<0.001$). Regarding specificity and sensitivity, BCL2 was less specific and sensitive in discriminating SCC and SCCIS from ISK (specificity, 20% and sensitivity, 16.0% and 30.0%, respectively)

Table (1): The clinical and histopathological difference between ISK ,SCCIS and SCC

	ISK	SCCIS	SCC
Risk factors	Inflammatory process	-prolonged exposure to solar radiation, -(HPV)16 ,18	-ionizing radiation -HPV
Site	head and neck	face and legs	Face, ears, scalp, dorsal hand
No of the lesion	• Solitary	single patch	single
Size	• less than 1 cm		Variable
Gross picture	• scalyskin colored papule with or without filiform growth	Large erythematous scaly plaque, which expands centrifugally	erythematous plaque, nodule, ulcer
ulceration, hemorrhage	• absent	absent	Present
Border	• circumscribed borders	irregular border	Infiltrative
squamous eddies	large number, small size and circumscribed configuration.	Absent	prominent Irregular ,variable sized and shaped eddies
cellular atypia	Not seen	full-thickness involvement of the epidermis , by atypical keratinocytes and disorganization	Prominent
Mitotic figure	Not seen	Present	scattered mitotic figures
Nuclear pleomorphism	Not seen	Present	enlargement of nuclei
Necrotic keratinocyttes	clustered necrotic keratinocytes within the lower epidermal layers	dyskeratotic cells present	
acantholysis	Absent	Absent	foci of lacy acantholysis
Dermal inflammation	lichenoid inflammatory infiltrate in the dermis	variable inflammatory response	

ISK: Irritated Seborrehic Keratosis**, SCCIS:** Squamous cell carcinoma in-situ **,SCC :** squamous cell carcinoma [**8**].

Table (2): summary of markers used in the study

Antibody	Type	Cat.No	Dilution	Positive control	incubation	Antigen retrieval
IMP3	Rabbit polyclonal	Novus biological	1:100	Fetal liver	1h	Citrate buffer Ph(6)
BCL2	Mouse monoclonal	Thermoscientific USA	1:200	follicular lymphoma	1h	EDTA Ph(9)

Table (3)**:immunohistochmical expression of BCL2 and > in studied cases**

Type	No	BCL2		P value	IMP3				P value
		Negative	positive	<0.01	0	1	2	3	<0.01
ISk	15	3(20%)	12(80%)		11 (73.5%)	2 (13.5%)	1 (6.5%)	1(6.5%)	
SCCIS	10	7 (70%)	3 (30%)		1 (10%)	1 (10%)	2(20%)	6(60%)	
SCC	25	21 (84%)	4 (16%)		1 (4%)	2 (8%)	2(8%)	20(80%)	
Total	50	31	19		13	5	5	27	

ISK : IrritatedSeborrehic Keratosis **,SCCIS :**Squamous cell carcinoma insitu **,SCC :** squamous cell carcinoma

Table (4) : Combined expression of IMP3 and BCL-2 among the studied cases

Sum of markers	ISK	SCCIS	SCC	Statistical test (FET)	P value
				32.79	<0.001**
N BCL2+ P IMP3	0(0.0)	7(70.0)	21(84.0)		
P BCL2 + N IMP3	8(53.3)	2(20.0)	1(4.0)		
Total	15	10	25		

ISK : Irritated Seborrehic Keratosis **,SCCIS :**Squamous cell carcinoma insitu **,SCC :** squamous cell carcinoma ,P :Positive ,N : Negative.

Table (5) : Diagnostic Performance for BCL2 and IMP3 in studied cases

	BCL2			IMP3		
	SCCIS versus ISK	SCC versus ISK	SCC versus SCCIS	SCCIS versus ISK	SCC versus ISK	SCC versus SCCIS
AUC	0.25	0.18	0.43	0.823	0.924	0.61
Sensitivity	30.0	16.0	16.0	80.0	96.0	96.0
Specificity	20.0	20.0	70.0	73.3	73.3	20.0
PPV	20.0	25.0	57.1	66.7	85.7	75.0
NPV	30.0	12.5	25.0	84.6	91.7	66.7
Accuracy	24.0	17.5	31.4	76.0	87.5	74.3

ISK : Irritated Seborrehic Keratosis **,SCCIS :**Squamous cell carcinoma insitu **,SCC :** squamous cell carcinoma , PPV positive predictive value**,** NPV negative predictive value

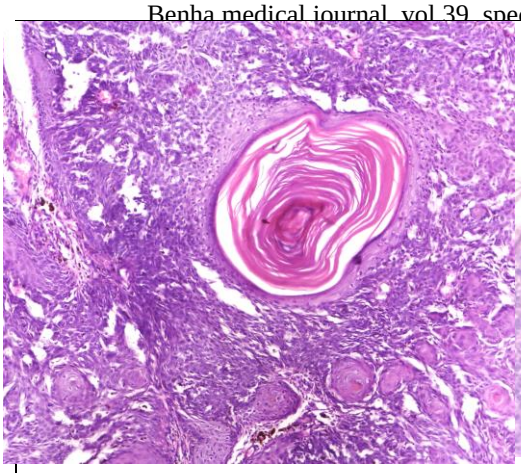
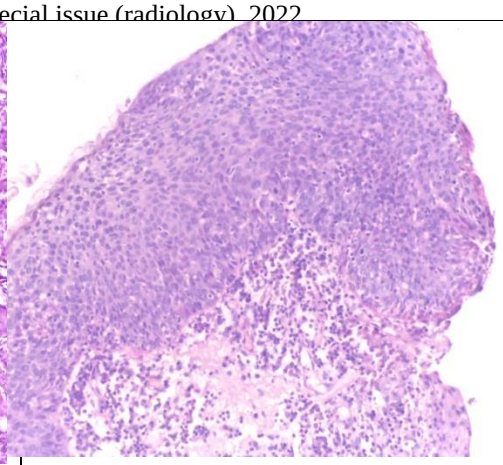
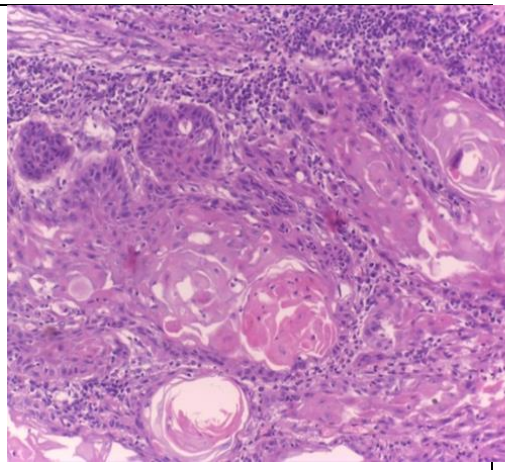
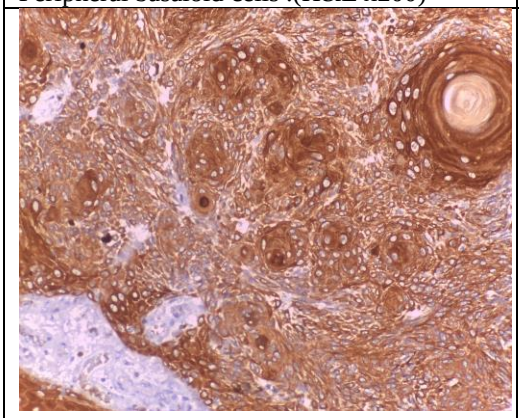
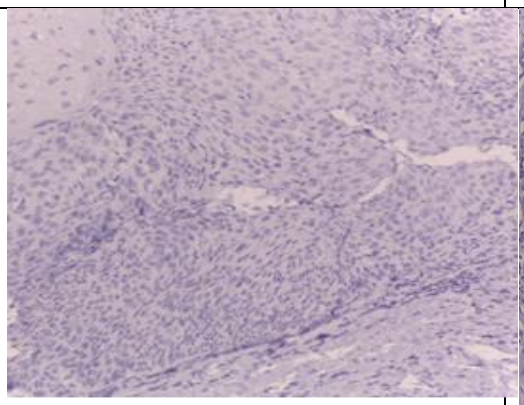
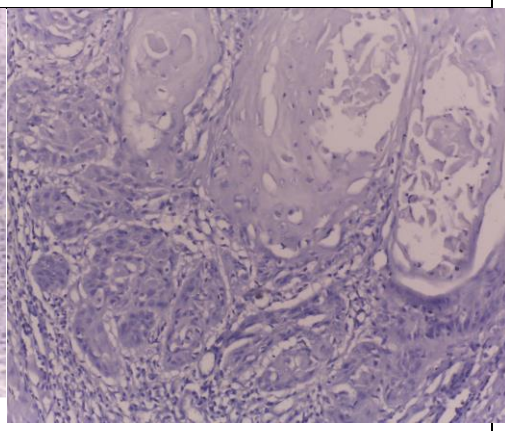
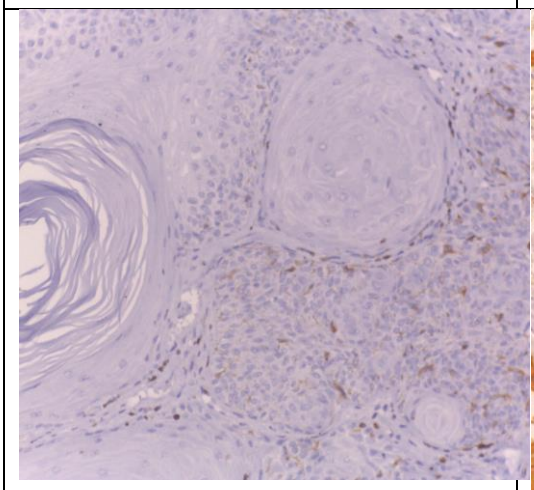
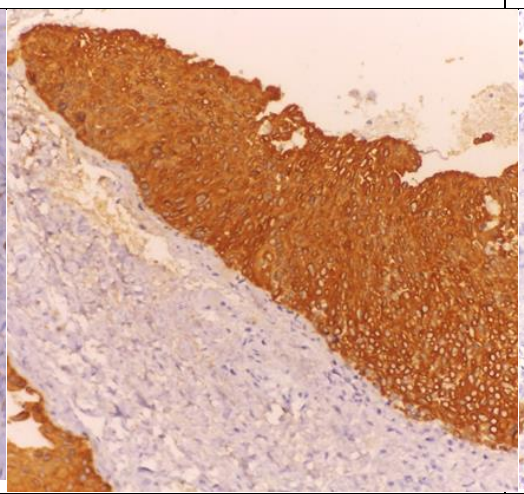
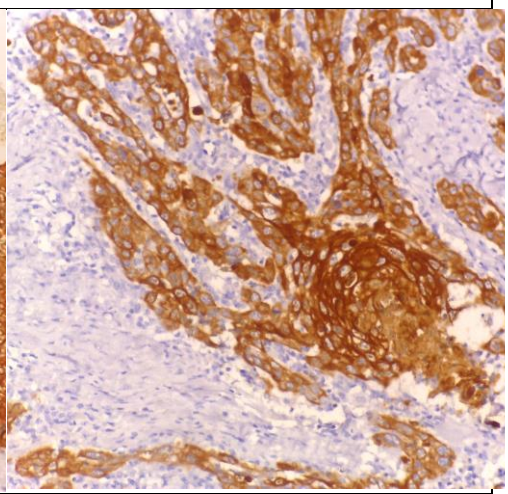
		

Fig (1) A- ISK showing Well circumscribed endophytic growth of squamous cells with lobular extension into the dermis showing Variable number of squamous eddies and Peripheral basaloid cells .(H&E x200)	B- SCCIS showing highly atypical pleomorphic epithelium involving the whole epidermal thickness (H&E X200)	C- SCC ,grade II showing nests of malignant squamous epithelium with central keratin pearls (H&E X200)
		
D- -Positive diffuse Bcl2 cytoplasmic expression in ISK (IHC x200)	E-Negative Bcl2 expression in SCCIS (IHC x200)	F-Negative Bcl2 expression in invasive SCC (IHC x200)
		
G-low IMP3 cytoplasmic expression in ISK (Ihc x,200)	H-Diffuse strong cytoplasmic expression of IMP3 in SCCIS (Ihc x,200)	I-Diffuse strong cytoplasmic expression of IMP3 in invasive SCC (Ihc x,200)

Discussion

ISK and SCC (both in-situ and invasive) are among the most common cutaneous tumors diagnosed on a daily basis in clinicopathological practice. Sometimes, ISK can be difficult to differentiate from both SCCIS and SCC at the microscopic level. Several studies have examined the pattern of expressionofIMP3and BCL2 in various cutaneous neoplasms, both benign and malignant [**11, 14**].

BCL2 is an antiapoptotic marker that plays a major role in regulating cell death. In the present study,BCL2was positively expressed in 80% of ISK cases compared with 30% and 16% of SCCIS and invasive SCC cases, respectively, showing a statistically significant positive correlation($p<0.01$).

In agreement with these results, other studies have reported the expression of IMP3 is an mRNA-binding protein is involved in normal embryonic development as well as tumorigenesis. It has been reported as highly expressed in SCC but negatively expressed in benign squamous epithelium lesions **[14,21,22,23]**Similarly, 90%of SCCIS and 96% of SCC cases were reported as IMP3-positive compared with 26.5% of ISK cases, and 80 of SCCIS and 88ofSCC cases were scored as +2or +3

BCL2 in almost all cases of SK, while 90%of SCCs were reported as negative or focally positive [**11, 17–19**]. However, [**18**] found that 67% of SCCs were weakly positive forBCL2 [**18**].This discrepancy may be due to differences in the IHC method, differences in the interpretation of the IHC results, or the diverse number of specimens.

In the current study, most of SCC and SCCIS cases were negative, for BCL2.In contrast to, BCL2 was frequently highly expressed in ISK cases. SK is thought to originate from basal keratinocytes which positively express BCL2.In contrast, SCC arises from supra basal keratinocytes that negatively express BCL2, and this may explain the altered staining patterns [**20**

In the current study, the combined IHC patternofIMP3 (+)/BCL2 (−)in invasive SCC and in SCCIS was significant, while an IMP3 (−)/BCL2(+)expression pattern was apparent in ISK($p<0.001$). Thus, these expression patterns may increases their diagnostic importance in discriminating SCC (both invasive and in-situ) from ISK. This is in agreement with the study done in 2018 by a group of researchers [**9**].

Conclusion

This study concluded that immunohistochemical detection of IMP3and/or BCL2 expression might be of diagnostic value in differentiating ISK from both invasive SCC and SCCIS. However, they do not play a significant role in distinguishing SCC from SCCIS. Further large-scale studies are necessary to approve or dismiss the divergent features of our findings.

References

1-Diepgen T. L and Mahler. V."The epidemiology of skin cancer," British Journal of Dermatology, vol. 146, Supplement, no. 61, pp. 1–6, 2002.

2- Swanson P. E, Fitzpatrick M. M, Ritter J. H, Glusac E. J, & Wick M. R.. "Immunohistologic differential diagnosis of basal cell carcinoma, squamous cell carcinoma, and trichoepithelioma in small cutaneous biopsy specimens," Journal of Cutaneous Pathology, vol. 25, no. 3, pp. 153–159, 1998.

3-Morton C. A, Birnie A. J, and D. J. Eedy, "British Association of Dermatologists' guidelines for the management of squamous cell carcinoma in situ (Bowen's disease) 2014," British Journal of Dermatology, vol. 170, no. 2, pp. 245–260, 2014.

4- Weedon D, Haneke E, Martinka M, Elgart G.W, Mortimore R.J, Gross C., et al.,Seborrhoeic keratosis,in: LeBoit, Philip E., ed. *Pathology and genetics of skin tumours*. Vol. 6. IARC, 2006 ; pp 41-43.

5-Busam KJ. 1st ed. Philadelphia: Elsevier; 2010. Dermatopathology: A volume in the series foundation in diagnostic pathology; pp. 336–343.

6-Phulari RG, Buddhdev K, Rathore R, Patel S. Seborrheic keratosis. Journal of oral and maxillofacial pathology: JOMFP. 2014 May;18(2):327.

7- Tan K.B, Tan S.H, Aw D.C.W, Jaffar H, Lim T.C, Lee S.J, et al., Simulators of squamous cell Carcinoma of the skin: Diagnostic challenges on small biopsies and clinicopathological correlation, J. Skin Cancer. 2013 ;752864, 10 pages.

8-Elder DE. 10th ed. Philadelphia: Lipincott; 2009. Lever's histopathology of the skin; pp. 795–798

9-Justin D. Richey | April C. Deng | Karen Dresser | Patrick O'Donnell, Kristine M. Cornejo :Distinguishing between irritated seborrheic keratosis and squamous cell carcinoma in situ using BCL-2 and IMP3immunohistochemistry . J CutanPathol. 2018;45:603–609.

10-Liu Y, Huo Z, Yan B, Lin X, Zhou Z.N, Liang X, et al. , Prolyl hydroxylase 3 interacts with Bcl-2 toregulate doxorubicin-induced apoptosis in H9c2cells,Biochem.Biophys. Res.Commun.401 (2010) 231-237.

11-Gaballah MA, Ahmed RA. Diagnostic value of CD10 and Bcl2 expression in distinguishing cutaneous basal cell carcinoma from squamous cell carcinoma and seborrheic keratosis.Pathol Res Pract. 2015; 211(12):931-938.

12- Yantiss R.K, Woda B.A., Fanger G.R , Kalos M , Whalen G.F, Tada H, et al. KOC (K homology domain containing protein overexpressed in cancer): a novel molecular marker that distinguishes between benign and malignant lesions of the pancreas.Am J SurgPathol 2005; 29: 188–95.

13-Xu H, Bourne PA, Spaulding BO, Wang HL. High-grade neuroendocrine carcinomas of the lung express K homology domain containing protein overexpressed in cancer but carcinoid tumors do not. Human pathology. 2007 Apr 1;38(4):555-63.

14- KANzAKI AK, KUdO MI, Ansai SI, Peng WX, Ishino K, Yamamoto T, et al. Insulin-like growth factor2 mRNA-binding protein-3 as a marker for distinguishing between cutaneous squamous cell

carcinoma and keratoacanthoma. Int J Oncol. 2016;48(3):1007-1015.

15-Wachter DL, Schlabrakowski A, Hoegel J, Kristiansen G, Hartmann A, Riener MO. Diagnostic value of immunohistochemical IMP3 expression in core needle biopsies of pancreatic ductal adenocarcinoma. Am J SurgPathol. 2011;35:873–877.

16- Yada K, Kashima K, Daa T, Kitano S, Fujiwara S, Yokoyama S. Expression of CD10 in basal cell carcinoma, Am. J. Dermatopathol.2004;26 (6):463-471.

17-Ko CJ, Shintaku P, Binder SW. Comparison of benign keratoses using p53, bcl-1, and bcl-2.J CutanPathol. 2005;32(5):356-359.

18-Stanimirovic A, Cupic H, Bosnjak B, Tomas D, Kruslin B, Belicza M. TP53, Bcl-2 and growth hormone receptor expression in cutaneous squamous cell carcinoma. ActaDermatovenerol Croat. 2005;13(4):201-205.

19-Ko CJ, Kim J, Phan J, Binder SW. Bcl-2-positive epidermal dendritic cells in inverted follicular keratoses but not squamous cell carcinomas or seborrheickeratoses. J CutanPathol. 2006;33(7):498-501

20-Puizina-Ivic N, Sapunar D, Marasovic D, Miric L. An overview of Bcl-2expression in histopathological variants of basal cell carcinoma, squamous cell carcinoma, actinic keratosis and seborrheic keratosis. Coll Antropol. 2008;32(suppl 2):61-65.

21-Li HG, Han JJ, Huang ZQ, Wang L, Chen WL, Shen XM. IMP3 is a novel biomarker to predict metastasis and prognosis of tongue squamous cell carcinoma. J Craniofac Surg. 2011;22(6):2022-2025

22-Chen K, Cornejo KM, Ye W, Wu Q, Liang J, Jiang Z. Oncofetal proteinIMP3: a new diagnostic biomarker for laryngeal carcinoma. Hum Pathol. 2013;44(10):2126-2131.

23- Soddu S, Di Felice E, Cabras S, Castellanos ME, Atzori L, Faa G,, et al. IMP-3 expression in keratoacanthomas and squamous cell carcinomas of the skin: an immunohistochemical study. Eur J Histochem. 2013;57(1):e6.

24- Duraiyan J, Govindarajan R, Kaliyappan K, Palanisamy M. Applications of immunohistochemistry. J Pharm Bioallied Sci. 2012;4(Suppl 2):S307-S309

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