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Lab Note

Vitamin D enzymes (CYP27A1, CYP27B1 and CYP24A1) and receptor expression in non-melanoma skin cancer

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Running title: Vitamin D enzymes and non-melanoma skin cancer

The relationship between vitamin D metabolic enzymes (CYP27A1, CYP27B1, and CYP24A1) and vitamin D receptor (VDR) in non-melanoma skin cancer (NMSC) development and progression is not entirely clear. However, several clinical studies and *in vitro* reports indicate a connection between vitamin D metabolic key players and NMSCs by demonstrating inhibitory effects on tumor cells and positive effects of higher circulatory 25-hydroxyvitamin D in skin cancer prevention [1]. Vitamin D synthesis is mediated via mitochondrial cytochrome P450 family hydroxylase enzymatic reactions: anabolic and catabolic hydroxylases (CYP27A1, CYP27B1, and CYP24A1). The importance of vitamin D mitochondrial enzymes in some cancers have been reported, however their role in NMSC requires further evaluation. CYP24A1 protein expression in lung adenocarcinoma patients was reported up to 50 folds greater compared to healthy lung and significantly higher in poorly differentiated cancers types. The survival was proportional to the level of CYP24A1 expression (42% survival with high CYP24A1 expression versus 81% survival with low level CYP24A1). The overexpression of CYP24A1 in lung cancer was concluded to have

an association with poor survival in lung adenocarcinoma patients. Since endogenous vitamin D degradation is tightly controlled by CYP24A1, the abrogation of vitamin D antiproliferative effects in lung cancers may be due to CYP24A1 overexpression [2]. It is clear that low circulatory vitamin D levels are linked to decreased VDR activity in endometrial and breast cancer, and deletion of VDR results in decreased tumor suppressor genes (*H19*, *HOTTIP*, *Nespas*, *Kcnq1ot1*, *lincRNA-p21*, *Foxn2-as*, *Gtl2-as*, and *H19-as*) and increased oncogenes (*mHOTAIR*, *Malat1*, and *SRA*). In fact, VDR suppresses oncogenes, induces tumor suppressor genes, inhibits cell proliferation and migration of malignant cells, and prevents metastasis and angiogenesis in cancer [3]. Interestingly, VDR single nucleotide polymorphisms have been linked to actinic keratosis (AK) development and poor survival of melanoma patients [4]. These reports indicated that polymorphisms in VDR resulted in less transcriptionally active VDR, with less efficient interactions with transcriptional factors and decreased growth inhibition by vitamin D [5]. It is suggested that VDR and vitamin D metabolic enzymes interact with tumor regulatory proteins in malignant cells, influencing tumor formation, growth and preventing angiogenic spread [6]. Hence, tumor promotion and progression is connected to VDR and its metabolic enzymes. Vitamin D hydroxylases may play an important role in tumorigenesis. Their functions extend beyond vitamin D synthesis and degradation. Reduced enzymatic activity of CYP27B1 is noted in aggressive types of melanomas [7]. CYP27A1 modulates VDR activation and plays a pivotal role in carcinogenesis. The preventative effects of high endogenous vitamin D levels in the development of neoplastic growth and its aggressiveness have been demonstrated. Metastatic types of NMSC are often associated with squamous cell carcinoma (SCC) lesions. However the most common type of NMSC is basal cell carcinoma (BCC) and AK known as a common precursor of SCC, which has the potential for metastatic spread [8]. BCC and SCC have important morphological differences and different metastatic potential. Whilst metastatic BCC is a less common condition, both BCC and SCC are malignant. Therefore, it is crucial to establish the status of intratumoral vitamin D enzymes and receptor.

Herein, the intratumoral expression of mitochondrial vitamin D enzymes and VDR in BCC, SCC, SCC *in situ* (SCCIS), AK and normal skin were determined by standard immunohistochemistry (IHC) tissue staining using biotinylated universal antibody streptavidin–biotin (LSAB+) System–HRP (K4063) and diaminobenzidine (Dako Botany, Australia). The primary unconjugated polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, USA) were used at the optimized dilutions (1:1000): goat anti-human CYP27A1, rabbit anti-human CYP27B1, rabbit anti-human CYP24A1, and rabbit

anti-human VDR. The secondary antibodies (Dako) were also used at their optimized dilutions (1:500): anti-mouse HRP, polyclonal rabbit anti-goat Ig, and polyclonal goat anti-rabbit Ig. All antibodies as supplied by the respective companies were characterized and validated for specificity by western blotting [9]. Tissue sections were received from the Department of Dermatology, St Vincent's Hospital (Melbourne, Australia) or purchased from Resolving Images Pty Ltd (Melbourne, Australia). In total, 20 BCCs, 15 AKs, 12 SCCISs, 8 SCCs and 5 normal skin tissues were stained from patients aged 41–93 years following informed consent and human ethics was obtained from the Department of Dermatology, St Vincent's Hospital and Victoria University, Australia. Detections of vitamin D metabolic enzymes (CYP27A, CYP27B1 and CYP24A1) and its receptors (VDR) in human NSK, AK, BCC, SCC and SCCIS are shown in **Figs. 1** and **2**. A summary of tissues stained with respective antibodies and expression levels and number of tissues positive are shown in **Table 1**. VDR showed uniform moderate to high expression in most tissues particularly in epidermal keratinocytes and to a lesser degree in keratinocytes of basal layers in AK, SCC and SCCIS. The expression of VDR in BCC is present in keratinocytes of all epidermal layers. In addition, VDR was shown to have a statistically significant association with CYP24A1 in all tumors, but not with NSK ($P<0.001$). CYP27A1 detection was very prominent in NSK, AK and SCCIS, with lower expression in some SCC and BCC. CYP27A1 expression was strongly associated ($P<0.05$) with CYP24A1 expression in BCC and SCCIS and almost reached significant association with CYP24A1 expression in SCC ($P=0.052$), and no association in NSK ($P>0.05$). CYP27B1 was expressed in NSK and most SCCIS with lower expression in most precancerous (AK) tissues and cancerous tissues (BCC and SCC). Inverse significant association was noted between CYP27B1 and CYP24A1 expression in all NMSC lesions, but not in NSK. The distribution of CYP24A1 expression was very different in all tumors and NSK. In AK, BCC, SCC and SCCIS there was intense CYP24A1 expression, whereas in most NSK tissues the expression of CYP24A1 was decreased. Specificity of the antibodies used on normal keratinocytes and malignant squamous keratinocytes (SCC-4 cells), and the effects of calcidiol and calcitriol on the expression of CYP27A1, CYP27B1, CYP24A1, and VDR on these cell lines were confirmed and assessed by western blotting (data not shown). The expression and correlation analysis of vitamin D metabolic proteins and VDR in tumor sections and normal skin was determined by IBM Statistical Package for the Social Sciences (SPSS) software using Multivariable Cross-tabulation Chi-Square test. Correlations were considered statistically significant with $P < 0.05$.

In summary, vitamin D metabolic enzymes and VDR expression in human NMSC were found to have statistically significant correlations amongst CYP27A1, CYP24A1 and VDR, but not CYP27B1. We present data that shows CYP27B1 to be diminished in NMSC, compared to normal skin, whilst CYP24A1 was elevated in most tumors, but not in normal skin. These findings are consistent with previous studies in ovarian carcinomas [10], where CYP24A1 overexpression in malignant cells was noted, whilst CYP27B1 expression was highly reduced.

We report crucial differences in the expression of CYP27B1 and CYP24A1 in normal and neoplastic tissues. These findings contribute to a better understanding of vitamin D metabolism in cutaneous carcinogenesis and could be used as an aid in the management of NMSC, particularly for further prognosis of disease recurrence.

References

1. Akutsu RL, Yolande Bastien, Alain Bestawros, Danny J. Enepekides, Martin J. Black, and John H. White. Regulation of Gene Expression by 1a,25-Dihydroxyvitamin D3 and Its Analog EB1089 under Growth- Inhibitory Conditions in Squamous Carcinoma Cells. Molecular Endocrinology 2001, 15: 1127-1139
2. Guoan Chen SHK, Amanda N. King, Lili Zhao, Robert U. Simpson, Paul J. Christensen, Zhuwen Wang, Dafydd G. Thomas, Thomas J. Giordano, Lin Lin, Dean E. Brenner, David G. Beer, and Nithya Ramnath. CYP24A1 Is an Independent Prognostic Marker of Survival in Patients with Lung Adenocarcinoma Clinical Cancer Research 2011, 17 817-826
3. Lopes N, Sousa B, Martins D. Alterations in vitamin D signalling and metabolic pathways in breast cancer progression: a study of VDR, CYP27B1 and CYP24A1 expression in benign and malignant breast lesions. BMC Cancer 2010, 10: 483
4. Morgese F, Soldato D, Pagliaretta S, Giampieri R, Brancorsini D, Torniai M, Rinaldi S, *et al.* Impact of phosphoinositide-3-kinase and vitamin D3 nuclear receptor single-nucleotide polymorphisms on the outcome of malignant melanoma patients. Oncotarget 2017, 8: 75914-75923
5. Carless MA, Kraska T, Lintell N, Neale RE, Green AC, Griffiths LR. Polymorphisms of the VDR gene are associated with presence of solar keratoses on the skin. The British journal of dermatology 2008, 159: 804-810

6. Vanoirbeek EK, A. Eelen, G. Verlinden, L. Bouillon, R. Feldman, D.Verstuyf, A. The anti-cancer and anti-inflammatory actions of 1,25(OH)2D3. Best Practice & Research Clinical Endocrinology & Metabolism 2011, 25: 593-604
7. Brożyna AA, Jóźwicki W, Janjetovic Z, Slominski AT. Expression of the vitamin D–activating enzyme 1α-hydroxylase (CYP27B1) decreases during melanoma progression. Human Pathology 2013, 44: 374-387
8. Poswar FO, Fraga CAC, Farias LC, Feltenberger JD, Cruz VPD, Santos SHS, Silveira CM, *et al.* Immunohistochemical analysis of TIMP-3 and MMP-9 in actinic keratosis, squamous cell carcinoma of the skin, and basal cell carcinoma. Pathology - Research and Practice 2013, 209: 705-709
9. Wierzbicka JM, Binek A, Ahrends T, Nowacka JD, Szydlowska A, Turczyk L, Wasiewicz T, *et al.* Differential antitumor effects of vitamin D analogues on colorectal carcinoma in culture. International journal of oncology 2015, 47: 1084-1096
10. Brozyna AA, Jozwicki W, Jochymski C, Slominski AT. Decreased expression of CYP27B1 correlates with the increased aggressiveness of ovarian carcinomas. Oncology reports 2015, 33: 599-606

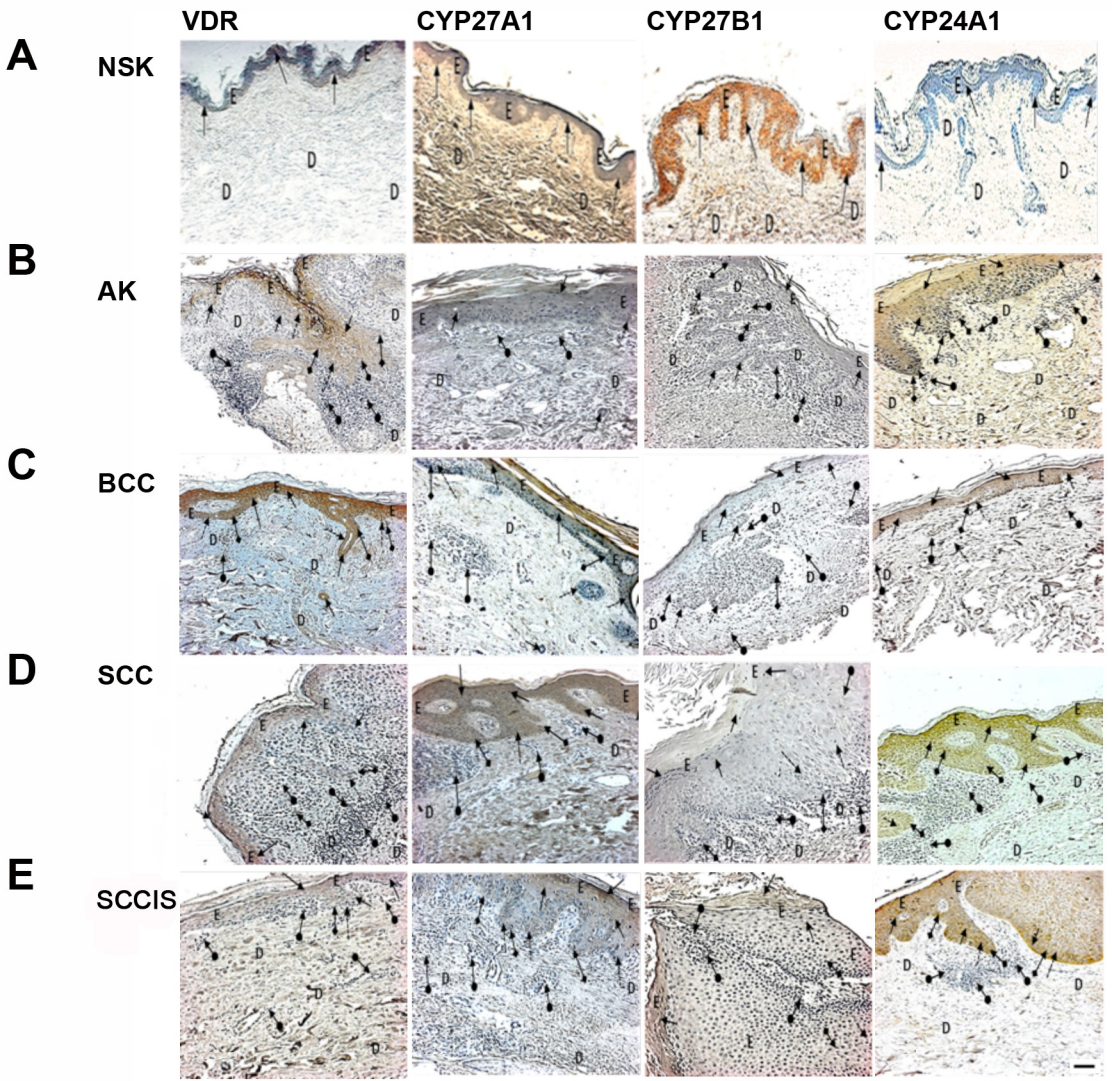
Figure Legends

Figure 1. Immunoperoxidase staining of skin tissues (NSK, AK, BCC, BCC, SCC, SCCIS) for the expressions of VDR, CYP27A1, CYP27B1 and CYP24A1 Disruptions of the basal membrane by neoplastic keratinocytes are indicated with ‘diamond-ended’ arrows. Immune cell infiltration is shown with ‘circled-ended’ arrows. The positively stained neoplastic, premalignant and normal keratinocytes are shown with solid black arrows and are indicative of the tissue staining intensity. Poorly differentiated keratinocytes are shown with black dashed arrows, where applicable. Scale bar: 20 μm. E, epidermal layer; D, dermal layer.

Figure 2. High magnification immunoperoxidase staining of skin tissues (AK, BCC, SCC) for the expressions of CYP27B1 and CYP24A1 At high magnification (x 400) in hyperproliferative and poorly differentiated keratinocytes minimal expression of CYP27B1

is noticeable. Elevated CYP24A1 expression is noted in AK and most BCC and SCC tissues. Scale bar: 50 μm.

Table 1.The expressions of VDR, CYP27A, CYP27B1 and CYP24A1 in NMSC tissues and normal skin


VDR
CYP27A1
CYP27B1
CYP24A1
A
NSK
B
AK
C
BCC
D
SCC
E
SCCIS

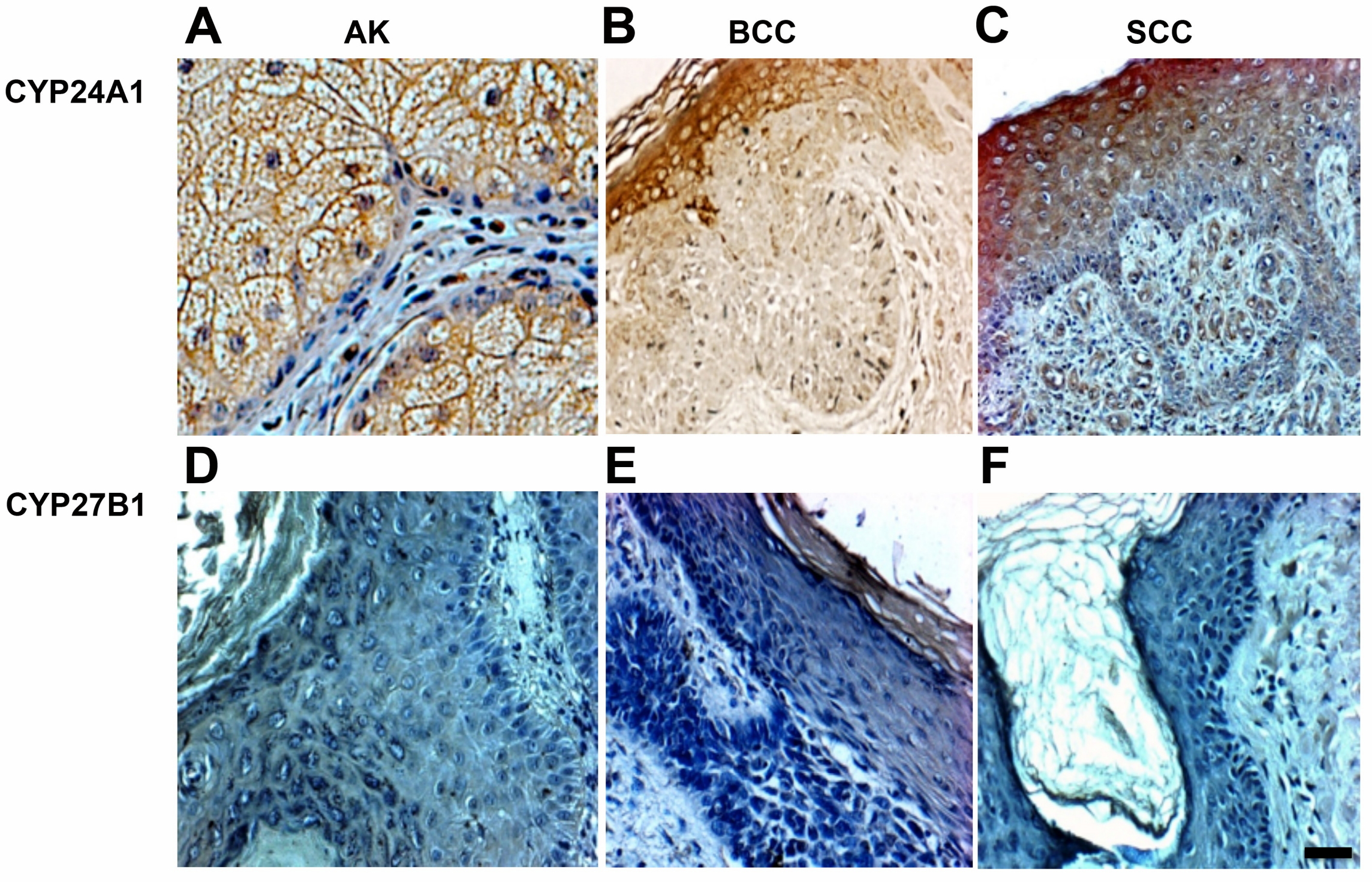

A
AK
B
BCC
C
SCC
CYP24A1
D
E
F
CYP27B1

Table 1. The expression of VDR, CYP27A, CYP27B1 and CYP24A1 in NMSC tissues and normal skin

Protein	**Low levels of expression (0-5%)**	**Moderate levels of expression (5-10%)**	**High levels of expression (> 10%)**
Normal skin			
Vitamin D CYP27A1 (CYP27A1)	0	0	5
CYP27B1 (CYP27B1)	1	1	3
Vitamin D3 24-hydroxylase (CYP24A1)	3	1	1
VDR	0	0	5
Actinic keratosis neoplastic lesions			
Vitamin D CYP27A1 (CYP27A1)	0	2	13
CYP27B1 (CYP27B1)	9	2	4
Vitamin D3 24-hydroxylase (CYP24A1)	0	0	15
VDR	1	2	12
Basal cell carcinoma			
Vitamin D CYP27A1 (CYP27A1)	5	6	10
CYP27B1 (CYP27B1)	12	5	4
Vitamin D3 24-hydroxylase (CYP24A1)	1	3	17
VDR	1	2	18
Squamous cell carcinoma			
Vitamin D CYP27A1 (CYP27A1)	2	4	2
CYP27B1 (CYP27B1)	4	4	0
Vitamin D3 24-hydroxylase (CYP24A1)	1	2	5
VDR	1	4	3
Squamous cell carcinoma *in situ*			
Vitamin D CYP27A1 (CYP27A1)	2	2	8
CYP27B1 (CYP27B1)	3	9	0
Vitamin D3 24-hydroxylase (CYP24A1)	1	2	9
VDR	2	2	8

Supplementary Table

The association of CYP24A1 with CYP27A1, CYP27B1 and VDR expression in NMSC; The multivariable Cross-tabulation Chi-Square analysis

CYP24A1	CYP27A1					CYP27B1					VDR				
	NSK	AK	BCC	SCC	SCCIS	NSK	AK	BCC	SCC	SCCIS	NSK	AK	BCC	SCC	SCCIS
Pearson Correlation R values	-0.802	0.708	0.691	0.750	0.875	0.563	-0.183	-0.383	-0.707	-0.93	0.698	-0.808 .	0.991	0.832	0.955
p values	0.096	0.03	0.001	0.052	0.0002	0.324	0.05	0.044	0.048	0.002	0.101	0.001	0.0001	0.001	0.001