P2X₅ and P2X₇ receptors in human warts and CIN 612 organotypic raft cultures of human papillomavirus infected keratinocytes

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Abstract

Purinergic receptors, which bind adenosine 5'-triphosphate (ATP), are expressed on human cutaneous keratinocytes and in squamous cell carcinomas. Studies on normal human epidermis and primary keratinocyte cultures have suggested that P2X₅ receptors are likely to be involved in keratinocyte differentiation and P2X₇ receptors are likely to be part of the machinery of end stage terminal differentiation/apoptosis of keratinocytes. P2X₇ receptor agonists can significantly reduce primary keratinocyte cell numbers in culture. Human papillomaviruses are increasingly recognised as important human carcinogens in the development of non-melanoma skin cancers. In our study, immunohistochemical analysis for P2X₅ and P2X₇ receptors was performed on paraffin sections of normal human skin, warts, raft cultures of normal human keratinocytes and raft cultures of CIN 612 cells, a model of keratinocytes infected with human papillomavirus type 31. In warts there was up-regulation of the expression of P2X₅ receptors. A similar pattern was seen in the CIN 612 raft cultures. Both P2X₅ and P2X₇ receptors were found in the nuclei of koilocytes, abnormal keratinocytes characteristic of human papillomavirus infection. P2X₅ and P2X₇ receptors may provide a new focus for therapeutic research into treatments for warts because these receptors can induce cell differentiation and cell death.

Abbreviations: ADP – adenosine 5'-diphosphate; ATP – adenosine 5'-triphosphate; BCC – basal cell carcinoma; DAB – diaminobenzidine; GFP – green fluorescent protein; HPV – human papillomavirus; NHEK – normal human epidermal keratinocytes; SCC – squamous cell carcinoma

Introduction

Non-melanoma skin cancer is the most frequently occurring malignancy worldwide in the Caucasian population [1]. Ultraviolet radiation is the major environmental factor in the pathogenesis of basal cell carcinomas (BCC) and squamous cell carcinomas (SCC), which tend to occur in sun-exposed sites [2]. The ratio of BCCs to SCCs is 5:1 in immunocompetent populations, whereas in immunosuppressed patients the ratio is reversed, with the risk of developing a SCC up to 250 times greater and the risk of developing a BCC 10 times greater than that in the general population [3]. The difference in incidence of these tumours in immunocompromised patients is thought to be due to the involvement of papillomaviruses. The association between warts and skin cancer was first noted in renal transplant recipients [4]. Human papillomaviruses (HPVs) are small double-stranded DNA viruses that are widespread in the human population. They are strictly epitheliotropic and infect only cutaneous and mucosal skin sites. Over 120 different HPV types have been identified, of which 80 have been characterised in full [3].

There is increasing evidence that purinergic signalling can have long-term, trophic effects in cell growth, proliferation, differentiation and death [5, 6]. Purinergic receptors are classified into two groups: P1 receptors are selective for adenosine and P2 receptors are selective for adenosine 5'-triphosphate (ATP) and adenosine 5'-diphosphate (ADP), which act as extracellular signalling molecules [7]. P2 receptors are sub-divided into P2X and P2Y receptor families [8, 9]. P2X receptors are ligand-gated ion channels, and are activated by extracellular ATP to elicit a flow of cations (Na⁺, K⁺ and Ca²⁺) across the plasma membrane. Seven subtypes of P2X receptors are recognised [10]. In contrast, P2Y receptors are G protein-coupled and eight subtypes of P2Y receptors have been described [11]. P2X receptors are largely viewed as mediators of short term, fast intercellular communication.

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Recent studies suggest that P2X receptors could also mediate trophic effects. P2X₅ receptors have been implicated in the regulation of osteoblastic differentiation and proliferation [12], and triggering the differentiation of skeletal muscle satellite cells [13]. P2X₇ receptors have been shown to mediate ATP-induced apoptosis [14, 15]. ATP is likely to be an important local messenger in the epidermis. Both P2X₅ and P2X₇ receptors are expressed on adult rat cutaneous keratinocytes and functional roles in the regulation of cell turnover have been proposed [16]. Studies on adult human epidermis and primary keratinocyte cultures [17] have suggested that P2X₅ receptors are likely to be involved in early keratinocyte differentiation and P2X₇ receptors are likely to be part of the machinery of end stage terminal differentiation/apoptosis of keratinocytes. P2X₅ and P2X₇ receptors have altered expression in both BCCs and in SCCs and functional experiments using a cutaneous squamous cell carcinoma cell line, A431, have shown that purinergic receptors could in future provide novel therapeutic targets in non-melanoma skin cancers [18]. P2X₅ receptors are also involved in the differentiation of human fetal epidermis [19] and in rat wound healing [20]. This study compares the distribution of P2X₅ and P2X₇ receptors in normal human skin, with that in human warts. Vegetative reproduction of HPV particles can only take place in highly differentiated keratinocytes. This has made it difficult to study the effects of HPV in vitro. We studied the distribution of P2X₅ and P2X₇ receptors in HPV infected keratinocytes grown as an organotypic raft culture, where the cells are permitted to differentiate and stratify. These receptors may be a useful target for further research into both HPV and the carcinogenesis of cutaneous SCCs using organotypic raft cultures of HPV infected keratinocytes as a research tool.

**Materials and methods**

**Tissues**

Paraffin sections of samples of normal skin, human warts, normal human epidermal keratinocyte (NHEK) raft cultures and CIN 612 (HPV 31 infected cell line) raft cultures were stained for P2X₅ and P2X₇ receptors. Ethics Committee Approval was obtained to harvest human skin samples. Paraffin blocks of warts, NHEK raft cultures and CIN 612 raft cultures were prepared by Roche Discovery, Welwyn. Six samples of each were used in the study, with at least four sections of each sample. P2X₅ and P2X₇ receptors were examined because they are involved in early keratinocyte differentiation and terminal keratinocyte differentiation/apoptosis respectively.

**Raft cultures of differentiated HPV-infected keratinocytes**

The method for raft culture of keratinocytes has already been described [21]. Briefly, primary human foreskin keratinocytes (NHEKs) were obtained from Clonetics (San Diego, USA), the CIN 612 cell line was established from a CIN I biopsy and contained HPV31b DNA. Epithelial cells were seeded onto collagen matrices containing J2 3T3 fibroblast feeders. When the epithelial cells had grown to confluence, collagen matrices were lifted onto stainless steel grids and the cells were fed by diffusion from under the matrix. The cells were allowed to stratify and differentiate at the air–liquid interface over a 16-day period. Raft cultures were then harvested, fixed in 4% paraformaldehyde and embedded in paraffin.

**Antibodies**

The immunogens used for production of polyclonal P2X₅ and P2X₇ antibodies were synthetic peptides corresponding to 15 receptor-type-specific amino acids in the intracellular C-termini of the cloned rat and human P2X receptors, as previously described [16, 22]. P2X₅ and P2X₇ antibodies were kept frozen at a stock concentration of 1 mg/ml and used at a dilution of 1:200.

**Immunohistochemical method for paraffin sections**

The method below was an adaptation of the routine method used for immunohistochemistry in paraffin sections at RAFT and was developed by Elizabeth Clayton, Histology Department, RAFT. The method is described in detail in [18]. Briefly, Microwave antigen retrieval was used for the visualization of both P2X₅ and P2X₇ receptors in 4 μm paraffin sections. P2X₅ receptors were demonstrated via tyramide amplification and a diaminobenzidine (DAB) final substrate system, so that receptors were stained brown. P2X₇ receptors were demonstrated using a routine Streptavidin Alkaline Phosphatase method and a Vector Red final substrate system (Vector Laboratories, Peterborough, UK), so that receptors were stained pink. Nuclei were counterstained blue with Harris’s haematoxylin. Control experiments were carried out with the primary antibody omitted from the staining procedure. From previous work in frozen sections, there was no staining in both the no primary controls and upon pre-absorption of the primary antibody with the corresponding peptide [17].

**Photography**

The results were analysed using a Zeiss Axioplan high definition light microscope (Oberkochen, Germany) mounted with a Leica DC 200 digital camera (Heerbrugg, Switzerland).

**Results**

**P2X₅ and P2X₇ receptors in paraffin sections of normal human skin**

P2X₅ immunoreactivity was present mainly in the viable cell layers of normal human skin (Figure 1a), where the
staining was confined largely to the cell membranes and the cytoplasm in epidermal keratinocytes, with occasional nuclear staining. P2X7 immunoreactivity was present in the epidermis of all normal skin samples, and was associated with cells and cell fragments in the stratum corneum (Figure 1b). There was some staining of the outermost edge of the stratum corneum with the P2X 5 receptor antibody, which was only slightly reduced in the no primary antibody control (Figure 1c), and therefore non-specific staining. There was no staining in the no primary control for the P2X7 receptor antibody (Figure 1d).

P2X5 and P2X7 receptors in paraffin sections of human warts

In warts, there was marked hyperkeratosis and parakeratosis within the stratum corneum. P2X5 receptors were present in nucleated keratinocytes in areas of parakeratosis, but not within the hyperkeratotic areas of the stratum corneum (Figure 2a). In contrast, P2X7 receptors were found in hyperkeratotic areas of the stratum corneum but not in parakeratotic areas of the wart (Figure 2b). P2X5 immunoreactivity was present in the majority of wart keratinocytes (Figure 2c), but few cells in the basal layer were positive, with most of the positively stained cells in the suprabasal layers. P2X7 receptors were found in the nuclei of suprabasal cells (Figure 2d). There was a prominent granular layer in the wart with koilocytes. Koilocytes are the characteristic cytological feature of HPV infection. Koilocytes are keratinocytes with pyknotic, deeply blue nuclei surrounded by a halo and clear cytoplasm with a paucity of keratohyaline granules. They usually indicate the presence of human papilloma virus. Both P2X5 and P2X7 receptors were found in the nuclei of koilocytes (Figure 2e,d). There was a band of heavy staining of the outermost edge of the stratum corneum with the P2X5 receptor antibody (Figure 2a), which was still present in the no primary antibody control (Figure 2e), and therefore was non-specific staining. There was no staining in the no primary control for the P2X7 receptor antibody.

P2X5 and P2X7 receptors in paraffin sections of raft cultures of normal human keratinocytes and of CIN 612 (HPV 31) cells

P2X5 immunoreactivity was present throughout all the layers of the raft cultures of normal human foreskin keratinocytes (Figure 3a), where the staining was confined largely to the cell membranes and the cytoplasm. P2X7 immunoreactivity was present in the raft cultures of normal human foreskin keratinocytes, staining weakly within the uppermost layer (rudimentary stratum corneum) (Figure 3b). The P2X7 receptor staining was not as strikingly positive as with the paraffin section of normal skin (Figure 1b).

P2X5 immunoreactivity was present in the CIN 612 (HPV 31) raft keratinocytes (Figure 3c), where the staining was seen within all layers of the raft. P2X7 immunoreactivity was present in the CIN 612 raft (Figure 3d) and was associated with the cell cytoplasm in the HPV infected cells. At higher power, the uppermost layers are highly disorganised, with nucleated cells at the surface of the raft (Figure 3e,f). There was positive staining in the cytoplasm of mitotic cells within the raft for both P2X5 (Figure 3e) and P2X7 (Figure 3f) receptors.
Discussion

This paper adds extra understanding of the role of P2X\textsubscript{5} and P2X\textsubscript{7} receptors in keratinocytes differentiating under abnormal circumstances, for example during human papilloma virus infection. Human papilloma viruses are increasingly recognised as an important human carcinogen and have been implicated in non-melanoma skin cancers [23, 24]. The association between skin warts and skin cancer was first noted in renal transplant recipients [4] who have a marked increase in susceptibility to both viral warts and non-melanoma skin cancer. Clinical and histological features of transplant SCCs indirectly support the progression of viral warts through increasingly dysplastic squamous lesions to invasive SCCs [25]. P2X\textsubscript{5} and P2X\textsubscript{7} receptors have altered expression in both BCCs and in SCCs [18]. We examined the expression of these receptors in human papilloma virus infected keratinocytes with the long-term aim of establishing whether they may also have a role as a potential therapeutic target.
In this paper changes in P2X5 and P2X7 receptor expression were examined within human papilloma virus infected differentiated keratinocytes in both warts and in a model system. The organotypic raft model was chosen because it would allow an in vitro model of both the normal epidermis and of a wart that could later be compared and manipulated with drugs. This model allows keratinocytes to differentiate and stratify at an air fluid interface. Since human papilloma virus replication tends to take place in differentiated keratinocytes, this model has the advantage of allowing us to study differentiated cells, which would be much harder to do with monolayer keratinocyte culture systems. Previous work has proposed that P2X5 receptors are involved in early differentiation of keratinocytes and that P2X7 receptors are likely to be part of the machinery of end stage terminal differentiation of keratinocytes. 2'- and 3'-0-(4-Benzoylemethyl) ATP, a potent P2X7 receptor agonist, causes a significant decrease in cell number via a direct effect on P2X7 receptors [17], which are also involved in mediating apoptosis [26, 27].

In normal skin, P2X5 receptors were found in the basal layer, stratum spinosum and weakly in the stratum granulosum, with occasional nuclear staining. There was little or no P2X5 receptor staining in the stratum corneum, apart from some staining artefact. In the raft cultures of normal human foreskin keratinocytes, P2X5 receptors were found throughout all layers, from the basal layer to the most differentiated layer. Warts arise because human papillomaviruses infect the basal keratinocyte of the epidermis, presumably through disruptions of the skin or mucosal surface [28]. The virus remains latent in basal cells as a circular episome. As keratinocytes differentiate and migrate to the surface, the virus is triggered to undergo replication and maturation. Hybridisation studies in situ of

Figure 3. Expression of P2X5 and P2X7 receptors in paraffin sections of raft cultures of normal human keratinocytes and of CIN 612 (HPV 31) cells. Nuclei were counterstained blue with haematoxylin. a P2X5 immunoreactivity (brown) was present throughout all layers of the raft cultures of normal human foreskin keratinocytes, where the staining was confined largely to the cell membranes and the cytoplasm. The raft culture was supported on a collagen matrix (C). Scale bar. 25 μm. b P2X7 immunoreactivity (pink) was present in the raft cultures of normal human foreskin keratinocytes, staining weakly within the uppermost layer (arrows). Scale bar. 25 μm. P2X5 immunoreactivity (brown) was present in the CIN 612 (HPV 31) raft keratinocytes, staining all layers of the raft. Scale bar. 50 μm. d P2X7 immunoreactivity (pink) was present in the CIN 612 raft and was associated with the cell cytoplasm and nucleus (arrow). Scale bar. 50 μm. e, f High power views of CIN 612 raft cultures: the uppermost layers are highly disorganised, with nucleated cells at the surface of the raft (arrows). There was also positive staining in the cytoplasm of mitotic cells (double arrows) within the raft for both e P2X5 receptors (brown) Scale bar 25 μm. and f P2X7 receptors (pink). Scale bar. 25 μm.
HPV lesions have shown that viral DNA synthesis occurs in the skin in the superficial stratum spinosum and full virus assembly with capsid production occurs in the stratum granulosum. In warts there is a prominent granular cell layer, within which there are vacuolated cells called koilocytes, characteristic of HPV infection. The process of virus replication alters the character of the epidermis, resulting in cutaneous or mucosal excrecences known as warts. In warts, P2X5 receptor staining was increased compared to that in normal skin. There were few P2X5 receptor positive cells in the basal layer, most of the positively stained cells being in the suprabasal layers.

HPV infections are classified into cutaneous, cutaneous involved in epidermodysplasia verruciformis, cutaneous and mucosal, and mucosal of low and high risk [29]. HPVs are linked with cervical cancer and SCC of the anus [30], which are associated with high risk genital HPV types 16, 18, 31, 33 and the low risk genital HPV types 6 and 11 [31]. Examination of immunostaining of P2X5 and P2X7 receptors on paraffin sections of cervical epithelium have shown that P2X5 receptors are expressed in the suprabasal, differentiated layers of the epithelium, but not in the basal layer. P2X7 receptor immunostaining was weakly present in the terminally differentiated cells of this non-keratinised epithelium. So these receptors are expressed in cervical cells. In the CIN 612 (HPV 31) raft cultures, P2X5 receptors were found in all cell layers and the level of staining was more intense than in the normal keratinocyte raft cultures.

Hyperproliferation is a feature of warts. Immunohistochemical labelling of frozen sections of other hyperproliferative lesions e.g. psoriasis, with P2X5 receptor antibodies has shown increased expression of the receptor in hyperproliferative areas of the epidermis. In psoriasis, rapid proliferation of keratinocytes leads to the production of immature keratin at the surface that has not completed its terminal differentiation process. This shows as silvery scaly psoriatic plaques on the skin surface. The receptor is again more prominent in suprabasal layers of the epidermis in differentiating and differentiated keratinocytes. This would suggest that this receptor is part of the differentiation process rather than part of a proliferative process. Interestingly a high prevalence of HPV DNA has also been found in psoriasis [32, 33].

P2X5 receptors have also been implicated in the regulation of osteoblastic differentiation and proliferation [12], and triggering the differentiation of skeletal muscle satellite cells [13]. In fetal rat skeletal muscle, P2X5 receptors are sequentially expressed during development [34]. P2X5 receptors are also involved in the differentiation of the human fetal epidermis [19] and play a role in wound healing in the rat epidermis [20].

In normal human skin, P2X7 receptor immunoreactivity was solely associated with cells and cell fragments within the stratum corneum [17]. In the raft cultures P2X7 receptor staining was very weak compared to normal skin. This may be due to the phenomenon of incomplete differentiation that occurs in raft cultures, thought to be associated with the presence of retinoids in the medium [35]. In warts, P2X7 immunoreactivity was associated with hyperkeratotic areas of the stratum corneum, as well as in nuclei of koilocytes in the suprabasal layers. The nuclei that were positive for P2X7 receptors were not normal: nuclei were shrunken, with much more intense, pink P2X7 receptor staining. In the CIN 612 raft cultures, P2X7 immunoreactivity was positive in both the cell cytoplasm and in the nucleus of HPV infected cells. The P2X7 receptor is a bifunctional molecule that can be triggered to act as a channel, permeable to small cations, or on prolonged stimulation form a cytolytic pore permeable to large hydrophilic molecules up to 900 Da [36]. The opening of this pore results in the increase in intracellular cytosolic free calcium ions and the induction of cell death [26, 27]. It is possible that the presence of P2X7 receptors in the nucleus of HPV infected cells indicates a severe disruption of the cellular machinery. It might be possible to use P2X7 receptor agonists to trigger apoptosis in these virally infected cells. P2X7 receptors are also found on dendritic cells, macrophages and microglial cells, where extracellular ATP can trigger apoptosis via these receptors and there is increasing evidence that this process is dependent on the caspase signalling cascade [14, 37].

In summary, P2X5 and P2X7 receptors may provide a useful focus for more research into new treatment modalities for warts and SCCs because these receptors can induce cell differentiation as well as cell death. Vegetative reproduction of HPV particles can only take place in highly differentiated keratinocytes. Raft cultures of both normal human keratinocytes and HPV infected cells could prove to be a useful tool for further study of these receptors in vitro.

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References

1. Miller DL, Weinstock DA. Nonmelanoma skin cancer in the United States: Incidence. J Am Acad Dermatol 1994; 30: 774–8.
2. Frost CA, Green AC. Epidemiology of solar keratoses. Br J Dermatol 1994; 131: 455–64.
3. de Villiers E-M, Laverge D, McLaren K, Benton EC. Prevailing papillomavirus types in non-melanoma carcinomas of the skin in renal allograft recipients. Int J Cancer 1997; 73: 356–61.
4. Walder BK, Robertson MR, Jeremy D. Skin cancer and immunosuppression. Lancet 1971; 11: 1282–3.
5. Abbracchio MP, Burnstock G. Purinergic signalling: Pathophysiological roles. Jpn J Pharmacol 1998; 78: 113–45.
6. Burnstock G. Purinergic signalling and vascular cell proliferation and death. Arterioscler Thromb Vasc Biol 2002; 22: 364–73.
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7. Burnstock G. A basis for distinguishing two types of purinergic receptor. In Straub RW, Bolis L (eds): Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach. New York: Raven Press, 1978; 107–18.

8. Burnstock G, Kennedy C. Is there a basis for distinguishing two types of P2-purinoceptor? Gen Pharmacol 1985; 16: 33–40.

9. Abbracchio MP, Burnstock G. Purinoceptors: Are there families of P2X and P2Y purinoceptors? Pharmacol Ther 1994; 64: 445–75.

10. Khakh BS, Burnstock G, Kennedy C et al. International Union of Pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. Pharmacol Rev 2001; 53: 107–18.

11. Burnstock, G. Introduction: ATP and Its Metabolites as Potent Extracellular Agents. In Schwiebert EM (ed): Current Topics in Membranes. Purinergic Receptors and Signalling. Vol. 54, San Diego: Academic Press, 1–27.

12. Hoebertz A, Townsend-Nicholson A, Glass R et al. Expression of P2 receptors in bone and cultured bone cells. Bone 2000; 27: 503–10.

13. Ryten M, Dunn PM, Neary JT, Burnstock G. ATP regulates the differentiation of mammalian skeletal muscle by activation of a P2X<sub>2</sub> receptor on satellite cells. J Cell Biol 2002; 158: 345–55.

14. Coutinho-Silva R, Persechini PM, Bisaglio RD et al. P2Z/P2X<sub>7</sub> receptor-dependent apoptosis of dendritic cells. Am J Physiol 1999; 276: C1139–47.

15. Di Virgilio F, Chiozzi P, Ferrari D et al. Nucleotide receptors: an emerging family of regulatory molecules in blood cells. Blood 2001; 97: 587–600.

16. Gröschel-Stewart U, Bardini M, Robson T, Burnstock G. Localization of P2X<sub>2</sub> and P2X<sub>7</sub> receptors by immunohistochemistry in rat stratified squamous epithelia. Cell Tissue Res 1999; 296: 599–605.

17. Greig AVH, Linge C, Terenghi G et al. Purinergic receptors are part of a functional signalling system for proliferation and differentiation of human epidermal keratinocytes. J Invest Dermatol 2003; 120: 1007–15.

18. Greig AVH, Linge C, Healy V et al. Expression of purinergic receptors in non-melanoma skin cancers and their functional roles in A431 cells. J Invest Dermatol 2003; 121: 315–27.

19. Greig AVH, Linge C, Cambrey A, Burnstock G. Purinergic receptors are part of a signalling system for keratinocyte proliferation, differentiation and apoptosis in human fetal epidermis. J Invest Dermatol 2003; 121: 1145–9.

20. Greig AVH, James SE, McGrouther DA et al. Purinergic receptor expression in the regenerating epidermis in a rat model of normal and delayed wound healing. Exp Dermatol 2003; 12: 860–71.

21. Ozbun MA, Meyers C. Transforming growth factor β1 induces differentiation in human papillomavirus-positive keratinocytes. J Virol 1996; 70: 5437–46.

22. Ogleby IB, Lachnit WG, Burnstock G, Ford APDW. Subunit specificity of polyclonal antiserum to the carboxy terminal regions of P2X<sub>2</sub> receptors, P2X<sub>2</sub> through P2X<sub>7</sub>; Drug Dev Res 1999; 47: 189–95.

23. Proby CM, Storey A, McGregor J, Leigh IM. Does human papillomavirus infection play a role in non-melanoma skin cancer? Papillomavirus Rep 1996; 7: 53–60.

24. zur Hausen H. Papillomavirus infections – a major cause of human cancers. Biochem Biophys Acta 1996; 1288: 55–78.

25. Blessing K, McLaren KM, Benton EC et al. Histopathology of skin lesions in renal allograft recipients: an assessment of viral features and dysplasia. Histopathology 1989; 14: 129–39.

26. Zheng LM, Zychlinsky A, Liu CC et al. Extracellular ATP as a trigger for apoptosis or programmed cell death. J Cell Biol 1991; 112: 279–88.

27. Ferrari D, Villalba M, Chiozzi P et al. Mouse microglial cells express a plasma membrane pore gated by extracellular ATP. J Immunol 1996; 156: 1531–9.

28. Cook MG, McKee PH. Infectious diseases. In McKee PH (ed) Pathology of the Skin. London: Gower Medical Publishers, 1989; 4.2–7.

29. Majewski S, Jablonska S. Human papillomavirus-associated tumours of the skin and mucosa. J Am Acad Dermatol 1997; 36: 659–85.

30. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002; 2: 342–50.

31. Gissman L, Boshart M, Durst M et al. Presence of human papillomavirus in genital tumours. J Invest Dermatol 1984; 83: 268–82.

32. Favre M, Orth G, Majewski S et al. Psoriasis: A possible reservoir for human papillomavirus type 5, the virus associated with skin carcinomas of epidermodysplasia verruciformis. J Invest Dermatol 1998; 110: 311–7.

33. Weissenborn SJ, Hopfl R, Weber F et al. High prevalence of a variety of epidermodysplasia verruciformis-associated human papillomaviruses in psoriatic skin of patients treated or not treated with PUVA. J Invest Dermatol 1999; 113: 122–6.

34. Ryten M, Hoebertz A, Burnstock G. Sequential expression of three receptor subtypes for extracellular ATP in developing rat skeletal muscle. Dev Dyn 2001; 221: 331–41.

35. Kopan R, Traska G, Fuchs E. Retinoids as important regulators of keratinization. J Cell Biol 1987; 105: 427–39.